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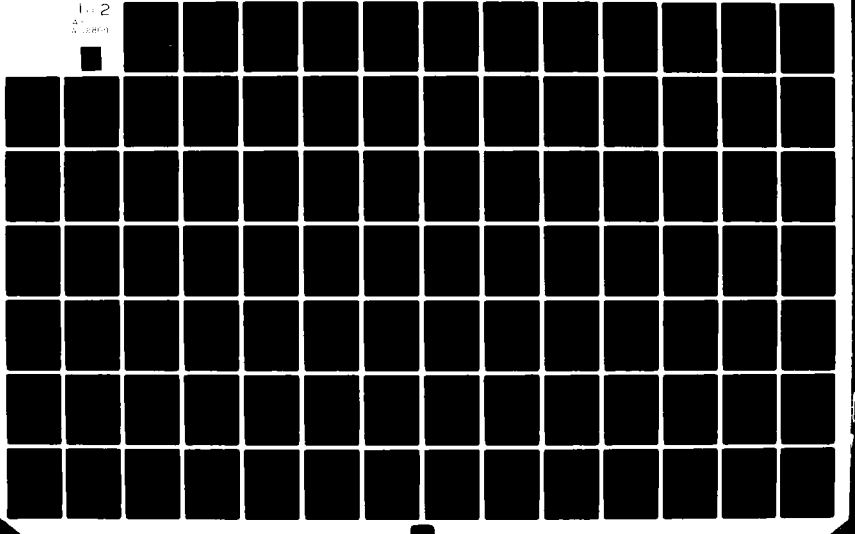

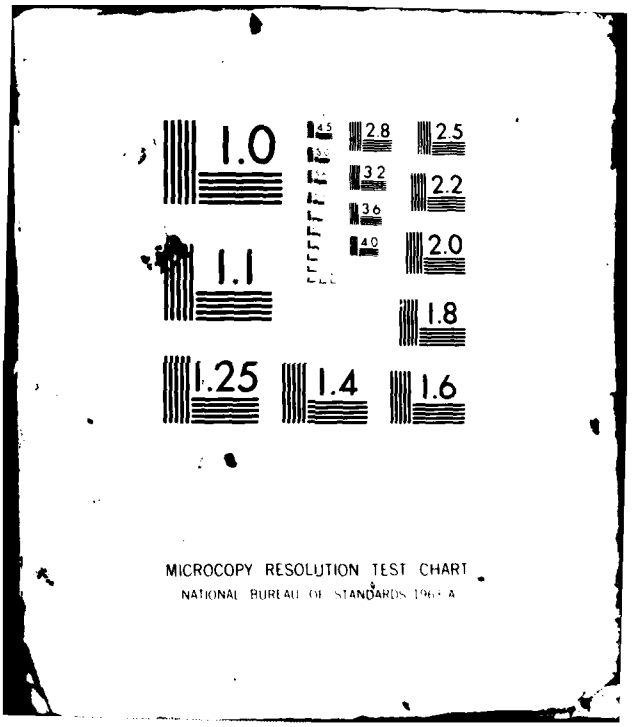
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MAMMALIAN TOXICOLOGY TESTING: PROBLEM DEFINITION STUDY

TECHNOLOGY CHANGES IMPACT ON TESTING REQUIREMENTS (U)

by
J. P. Glennon and R. J. Davenport

January, 1981

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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Contract DAMD17-81-C-1013

Life Systems, Inc.
Cleveland, OH 44122

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A task was completed to project the impact of technology changes on toxicology testing requirements. A group of thirteen experts in a broad range of toxicology-related disciplines evaluated for changes expected. Written summaries were prepared and included with this report.

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Report Subtitle	Life Systems, Inc. Report Number
Final Reports--	
Part 1. Comparative Analysis Report	LSI-TR-477-2
Part 2. Facility Installation Report	LSI-TR-477-3
Part 3. Impact of Future Changes Report	LSI-TR-477-4

FOREWORD

Reports for this Contract, DAMD17-81-C-1013, consist of three major final reports and twelve supporting documents. The Contract title, MAMMALIAN TOXICOLOGY TESTING: PROBLEM DEFINITION STUDY, is the main title for all the reports. Individual reports are subtitled and referenced with Life Systems, Inc. report numbers as detailed below. Please note that the Life Systems report numbers in test references are shortened. In the Defense Technical Information Center (DTIC) data base the reports are identified by the complete report numbers (i.e., LSI-TR-477-XXX) and complete numbers must be used for retrieval.

Report Subtitle	Life Systems, Inc. Report Number
Final Reports--	
Part 1. Comparative Analysis Report	LSI-TR-477-2
Part 2. Facility Installation Report	LSI-TR-477-3
Part 3. Impact of Future Changes Report	LSI-TR-477-4
Supporting Documents--	
Technology Changes Impact on Testing Requirements	LSI-TR-477-14
Quality Assurance Plan	LSI-TR-477-17A
Capability Modules	LSI-TR-477-19B
Technical Plan	LSI-TR-477-20A
Equipment Plan	LSI-TR-477-21A
Personnel Plan	LSI-TR-477-23A
Inhalation Chambers and Supporting Equipment Survey	LSI-TR-477-26A
Equipment List for Modules	LSI-TR-477-28B
AMTR Protocol/Pricing Report	LSI-TR-477-29A
Global Army Toxicology Requirements	LSI-TR-477-31A
Comparison Toxicology Test Costs	LSI-TR-477-36A
Annual Testing Capacity	LSI-TR-477-38A

SUMMARY

A study was completed to forecast potential regulatory and toxicology technology changes during the period from 1981 to 1990 and to evaluate the impact these changes may have on future toxicology testing resources. Supporting this study was a series of reports prepared by toxicology experts familiar with toxicology testing requirements and potential technology changes.

The present report provides the individual reports by a group of thirteen individuals.

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INTRODUCTION

A program was completed to define the Army's applied mammalian toxicology testing/research requirements. To the study was added tasks to define the type of Facility to meet these requirements and a task to project the impact of technology changes on toxicology testing requirements. This report addresses part of the later efforts.

APPROACH

Experts in animal husbandry, behavioral effects, biochemistry, biostatistics, epidemiology, general toxicology, genetics, inhalation toxicology, mutagenicity, neurotoxicology and oncology were asked to provide written summaries of their projections.

RESULT

The present report contains in its Appendix the written reports from the team forecasting toxicology technology changes.

CONCLUSION

Changes in the technology of toxicology will be associated with exposure assessment and studies of exposure routes, toxicology testing protocols, procedures and facilities, human studies, quality assurance, applied toxicology testing and interpretation of toxicology data. The utilization of a group of experts with differing toxicology backgrounds and related disciplines, offered an unique method for gaining insight into changes projected over the next decade that will influence toxicology testing.

APPENDIX 1

TASK ASSIGNMENT REPORTS

Report ID No.	Authors	Page Nos.
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TR-477-14-2

FORECAST OF POTENTIAL TECHNOLOGY CHANGES THAT MAY IMPACT TOXICOLOGICAL TESTING REQUIREMENTS

ICAIR Task Assignment No.: 107
Task Assignment Title: Impact of Changes

Task Assignment Report

by

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January, 1981

IMPACT OF CHANGES

The following questions are answered for their relevance to genetic toxicology with emphasis on chemical dosimetry.

1. Forecast equipment needs for laboratory toxicological testing.

   Mutagenic tests used in genetic toxicology can be grouped as follows: (1) bacteriological; (2) tissue culture; (3) *in vivo* insect (*Drosophila melanogaster*); (4) *in vivo* mammalian. While further development and refinement is continuing in all areas, the basic equipment for each area of testing is fairly fixed at this time, and I do not anticipate great changes in equipment required for any of these areas. However, there may be differences in emphasis of these areas. The major change that I anticipate is in a more quantitative approach leading to risk estimation, a change which requires improved dosimetry. Techniques using tracers labeled with radionuclides should become increasingly important in chemical dosimetry and should lead to equipment changes requiring greater emphasis on molecular dosimetry (Aaron and Lee, 1978, and Lee, 1978).

2. Forecast changes in analytical chemistry required for support of toxicological testing.

   The characteristic of genetic toxicology is that mutations can be induced at any time in the life cycle in germ cells and persist until transmittal to future generations. Therefore, exposure over a reproductive lifetime (approximately 30 years for man) is the important estimate to be derived from analytical chemistry methodologies. The Nuclear Regulatory Agency requires accumulative records so that a lifetime exposure record for any employee is available for those exposed to ionizing radiation. I anticipate that there will be a demand for analytical procedures for detecting low chronic levels of exposure in order to prepare lifetime exposure records for employees exposed to chemical mutagens.

3. What is the impact of advances in statistical and mathematical approaches to toxicological testing requirements (experimental design and data evaluation)?

   Recent reports in the Gene-Tox Program of EPA for the two test systems for *in vivo* germ cell mutagenesis (the mouse and *Drosophila melanogaster*) concentrated on the number of tests necessary to permit a negative decision with 5% confidence limits when the criterion was a fixed increase in the induced mutation frequency. (The EPA Gene-Tox reports should be published within a year.)

4. What is the impact of computers and data acquisition equipment on toxicology testing?

   Two areas are being affected by the improved computer capabilities: (1) Tables with greater accuracy for statistical tests are available and are being constructed in the Gene-Tox Program to determine the size of the test necessary for a negative decision; (2) The computers are used as access to data banks, for example, EMIC and Tox-line systems. Further extension of the data bases in these systems is anticipated.

5. Under the assumption that toxicological testing requirements will become more stringent (greater quality assurance), what will be the future value of toxicological data developed under present day standards?

   Some of the genetic test systems in mutagenesis have been developed and widely used for a long time. Examples are the sex-linked recessive lethal test in Drosophila and the specific locus test in the mouse. Criteria for these tests have been long established and, in the case with the sex-linked test in Drosophila, the committee upon which I served as chairman found acceptable the data collected as long as thirty years ago if the numerical data were included in the table so that the data could be submitted to current statistical tests. In contrast, other mutagenicity tests, such as tissue culture systems, have only recently been developed and there is still considerable disagreement among investigators as to the appropriate requirements for a valid test; therefore, further change in these systems may cause present data to be suspect.

6. Will the centralized data base storage and retrieval mechanisms that are established or being developed permit any significant reduction in toxicological testing requirements in the future?

   The number of substances tested for mutagenesis has increased rapidly in recent years and every indication suggests there will continue to be an increase in mutagenicity testing; therefore, a central data base becomes increasingly important in preventing unnecessary duplication of research and in comparing results among laboratories.

7. What will be the future impact/role of epidemiology studies in human health hazard assessments? (Will confirmatory human data always be required to supplement results from animal tests?)

   Ionizing radiation, known as a mutagen since 1927, has been extensively studied since the formation of the AEC in the late 1940's. However, extensive use of epidemiological methods have not yielded any positive results from ionizing radiation. Therefore, we cannot anticipate that epidemiology will be useful in the study of chemical mutagens. Genetic effects are delayed one or more generations, thus complicating the epidemiological approach for genetic effects.

8. What will be the projected speed for acceptance of technology changes for toxicity testing in your specific discipline area?

   Risk estimation of genetic hazard was required by law in the TSCA and FIFRA Acts and in the regulations of the pesticide program of EPA. However, at the time of these laws and regulations there was no successful estimation of genetic risk of a chemical, only that of ionizing radiation. I feel that risk estimation for chemicals that alkylate DNA can be now carried out, and I anticipate that they will be rapidly used to meet legal requirements. At the present time we cannot estimate genetic risk of substances whose products following reaction with DNA are unknown. The acceptance in terms of acts of law has run ahead of science in this particular area; therefore, rapid acceptance is anticipated.

9. Forecast the level of support for toxicology research and development, especially in the areas of basic research and its impact on technology advancement.

There seems to be a growing opinion in the U.S. to base levels of support on the state of the economy rather than upon specific needs; therefore, the level of support of basic research is likely to fluctuate relative to general economic conditions and to foreign incidents rather than to specific needs within the scientific community. Accurate forecasting of funding levels for basic research seems quite impractical.

10. What federal agency/organization programs are likely to be the pace setters for developing advances in toxicological testing technology, i.e., National Toxicology Program, EPA, National Academy of Sciences, etc.?

The pesticide programs of EPA under the FIFRA Act are the most advanced at this time for doing risk benefit analysis where the risk due to genetic hazard is considered. Reorganization and changes within EPA could drastically change the current set up.

11. Forecast the impact of mutagenic screening and other screening techniques on overall toxicology testing requirements.

Mutagenicity testing has two roles to play in general toxicology. One is the estimation of genetic risk due to mutagens induced in germ cells. The other is to give priority to testing for long-term carcinogens because of the correlation between tests for mutagens and tests for carcinogens. Considerable material has been written about the correlation between different mutagenicity screens and carcinogenesis. I would like to add one additional piece of information. The sex-linked recessive lethal test in Drosophila was developed without any thought of a carcinogenic screen, as it is strictly a test for mutations induced in germ cells; however, the recent results of the Gene-Tox Program showed a remarkably high correlation between the sex-linked recessive lethal test for germ cell mutations and carcinogenic tests for somatic cells in mammals. This correlation, in spite of the very separate history in development of these test assays, strongly suggests a fundamental correlation between carcinogenicity and mutagenicity.

12. What is the anticipated pace at which these screening techniques are likely to receive full acceptance as the basis for regulatory actions?

Pesticide programs of EPA under the FIFRA Act already use positive results in two submammalian mutagenicity screens as sufficient evidence for a rebuttable presumption against registration. Therefore, a place in regulatory activity has already been found for mutagenicity screens.

13. Will concern for synergistic effects due to exposures to multiple chemicals significantly impact short- and/or long-term toxicological testing requirements?

For low level chronic exposure where only the linear component (i.e., one hit event) is used in risk estimation, it is not anticipated that true synergism (i.e., departure from additivity) can be observed; therefore,

synergism should not be a problem in estimating risk for low level exposures. For high level acute exposures, as might occur as a result of an accident, multi-hit events and chromosomal aberrations requiring multi-hits could be important. Therefore, the total summation of conditions at the time of an accidental high level exposure may be critical in evaluating the risk resulting from an accident.

14. Will there be a trend toward the consolidation of toxicology testing protocols?

    Yes. The TSCA Act makes it mandatory that there be a consideration of standardization requirements for test protocols among different Federal agencies; however, different types of mutagenicity tests (i.e., tissue culture vs. Drosophila) require differences in points of concern, so in the Gene-Tox Committee we found it impractical to use even the same form in extracting data for cellular and in vivo test systems.

15. What is/would be the impact of focusing on the toxicological properties of chemical groups as opposed to specific chemical compounds?

    It is hoped that by proper grouping of chemical compounds and be testing thoroughly representatives of each group that it will not be necessary to test each member of the group, thereby substantially increasing the efficiency of genetic tests.

16. What progress related to cancer research would impact toxicological testing requirement, i.e., in areas of defining specific causes for cancers, or in the treatment and "cure" of cancer?

    This question is not within the areas of my expertise.

17. Forecast the impact and pace of developments in improved risk assessment techniques.

    Estimates of genetic risk due to ionizing radiation have been made for over a quarter of a century. However, application to chemical mutagens requires the development of chemical dosimetry along lines parallel to those of ionizing radiation dosimetry.

18. Forecast the role of structure activity relationships as they may replace certain toxicity testing requirements.

    This question should be grouped with question 15 in that if the grouping is proper, one would anticipate that it should not be necessary to test in detail all of the compounds in the group, but only representative samples.

19. Forecast the role/impact of improved radiolabeling techniques and chemistry (analytical and clinical) on toxicological testing requirements.

    Labeling with radionuclides enables the investigator to measure levels of alkylation of DNA induced by exposures comparable to those encountered by man in his environment. Therefore, it is not necessary to extrapolate from very high exposure level experiments to low level that man may encounter, if the proper dosimetry is done with chemical mutagens labeled

with radionuclides at a high specific activity. I anticipate the major change in equipment in mutagenicity testing to be the improved sensitivity of radiotracer techniques.

20. Evaluate the availability of laboratory animals on toxicology testing technology and/or requirements (controversies associated with use of dogs, scarcity and expense for use of primates, etc.).

    The standard test for point mutations in the mammal, the specific locus test, requires so many animals at such high cost for average to weak mutagens that it is completely impractical to consider testing all chemical mutagens by this means, either from the standpoint of availability or cost. Therefore, grouping by chemical activity and testing only a sample of each group in the mouse specific locus test will be necessary. A greater reliance on nonmammalian test systems is essential to cover the large number of chemical mutagens. The use of relatively large animals like dogs is possible with radioactive labeled compounds in order to test the distribution to the germ line following various routes of exposure. Only a relatively small number of animals are required for tests with radionuclides.

21. Are advances/standardization of neurotoxicity and behavioral effects testing believed to take place in the relatively near future so that these types of effects will have greater acceptance as a basis for establishing rules and regulations?

    I am not sufficiently experienced in neurotoxic testing to answer this question.

22. Forecast the availability of scarce personnel (e.g., veterinary pathologists) on the ability to perform toxicity testing.

    Our system of higher education is capable of producing large numbers of highly trained, technical personnel through graduate and postgraduate programs if sufficient, sustained funding is available.

23. Evaluate the role of scientific/professional societies in promoting technology changes that will impact testing requirements.

    The Environmental Mutagen Society had has a significant role in promoting genetic toxicology.

24. What will be the influence of current basic research investments on future testing technology?

    Developing the proper grouping of chemicals so that complete genetic testing is not necessary for each member of a group will have considerable economic impact upon genetic testing.

25. What are the best analogies to toxicity testing technology?

    For genetic toxicology the best analogy for chemical mutagens is the extensive work that has been done in ionizing radiation.

26. Forecast the degree of concern for the safety and health of persons performing toxicity testing and what impact this will have on future testing resource requirements.

The view that an unknown compound is hazardous until proven safe is replacing the view that a compound is safe until proven hazardous; therefore, maximum protective characteristics, involving glove boxes, hood, etc., are to be assumed as necessary equipment in genetic toxicology work.

27. What impact will medical treatment/advances have on reducing the concern with certain adverse toxic effects?

Because of the high cost of medical treatment, there is not likely to be any reduced concern in mutagenic agents due to improved methods of treatment; furthermore, successful treatment of a medical disorder maintains the mutant gene in the population, and therefore, requires continued medical expenditures through successive generations.

28. Will there be increased or decreased emphasis placed on toxicological effects which are reversible or irreversible?

Genetic toxicology deals only with the irreversible effects.

29. What technology changes are anticipated in the areas of routes of exposure: (a) inhalation, (b) oral, (c) dermal, (d) ocular, (e) other?

Chemical dosimetry of chemical mutagens should be done for all relevant levels of exposure. Only a relatively few animals are required in the chemical dosimetry experiments. Once the chemical dosimetry has properly determined the dose to the germ cells, the genetic effect and mutagenic risk can be assessed using any route of exposure, providing the dose to the germ cells is accurately determined.

30. What technology changes are anticipated in the area of animals used for toxicology testing: (a) rodents, (b) primates, (c) other animals?

As stated above, the large number of mutagens to be tested coupled with the large number of animals required for in vivo mammalian testing requires a dependence upon nonmammalian test systems for genetic toxicology. Hopefully, a representative of each major group of chemical mutagens will be tested in the in vivo mammalian system.

31. What advances are likely in non-animal testing that will reduce the amount/extent of animal testing?

The use of the non-animal testing seems non-scientific to me. I would suggest that extensive use of nonmammalian systems is going to be required because of the limitations on size of the mammalian germ cell tests. These nonmammalian test systems can include in vivo germ cell tests with insects, i.e., the sex-linked recessive lethal test in Drosophila melanogaster, somatic cell genetics, and bacterial tests.

32. What technology changes are anticipated in the area of duration of toxicology testing studies, (a) for acute effects, (b) for subchronic/subacute effects, (c) for chronic effects?

There should a greater emphasis on dosimetry studies of chronic, low level exposures in order to duplicate the physiological conditions under which man is exposed to most chemical mutagens.

33. Will it be required to duplicate the route of actual human exposure during future toxicity studies with animals?

Dosimetry studies to the germ cells should be conducted with mammals using the actual route of exposure by which man is to be exposed. Genetic tests may then be conducted by determining the dose to the germ line and by using any convenient route of exposure.

34. Will it be possible to use non-inhalation toxicology data to predict human health hazards associated with inhalation exposures? Is this due primarily to economic constraints or is it likely to be a technically "acceptable" alternative?

Inhalation toxicology is not my area of expertise; however, by dividing into two separate experiments (1) the relation of exposure to dose to the germ cells and (2) the relation of dose to the germ cell to mutation frequency, it is then possible to study the route of exposure with very few animals per experiment. The genetic experiment which requires a large number of animals is performed only once with the exposure by an convenient route. By this approach the study of the effects of different routes of exposure is not as prohibitively expensive as would be the case if one combined route of exposure with studies of mutation frequency in the same experiment.

35. Forecast potential advances in data evaluation and interpretation techniques that may permit improved communication of the results from toxicity testing to both decision makers and the general public (i.e., will the creditibility of the scientific community to predict human health hazards inprove, deteriorate or remain at its present level).

The use of radionuclides to permit chemical dosimetry for levels of exposure comparable to those man may encounter coupled with determination of the dosage response curve with reliable genetic test systems should make the prediction of genetic health effects more reliable in the future.

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TR-477-14-3

FORECAST OF POTENTIAL TECHNOLOGY CHANGES THAT MAY IMPACT TOXICOLOGICAL TESTING REQUIREMENTS

ICAIR Task Assignment No.: 107
Task Assignment Title: Impact of Changes

Task Assignment Report

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January, 1981

IMPACT OF CHANGES

1. Will toxicological testing requirements likely become more stringent? Will toxicological data developed under present day standards have future value in regulatory decision making?

   The Good Laboratory Practices Regulations (GLP's) have forced the toxicological testing laboratories to develop higher standards of operation. In addition to the improvement of physical facilities and use of computerized systems for identifying and assembling the data of a toxicological study, stricter requirements will continue to evolve to allow more precise control of protocol parameters for a particular test procedure. Although GLPs increase the requirements for assurance systems that can be readily evaluated by statistical means, there are many examples of research data which have proved valuable in providing useful information about a material, althugh the test parameters used would not meet the GLP standards of today. If the earlier-derived data is to be given adequate consideration, however, there will be a need for experienced scientists to use mature judgment in evaluating such data for regulatory purposes.

   The forecast here is that "earlier developed data," if it has the background of an adequately planned study, and if the data can be supported, will be used in the future to guide regulatory decisions. However, critical and controversial materials will, indeed, require studies to be designed and conducted along the parameters of current GLPs at the time such studies are undertaken.

2. What role will centralized data based storage and retrieval mechanisms play in toxicological testing requirements in the future?

   A principal benefit from the further development of computerized data bases will be the growth of base line information which will be useful in comparing data from a current study to historical background. It is not expected that any of the principal efforts concerned with toxicological testing will be significantly reduced by the increased use of centralized data bases. It is likely that there will be a gradual increase in the acceptance of centralized data bases, wherein information from diverse toxicological studies will be available for reference.

3. What will be the future role of epidemiological studies in human health hazard assessments?

   Epidemiology, as a discipline, will grow and become an increasingly important means of gathering toxicological information on human populations. It is also expected that there will be greater efforts to develop "base line" information on populations, in anticipation that such data may be useful in future epidemiological surveys.

   It is expected that epidemiology as an investigative tool will continue to grow and be increasingly important in assessing toxicological effects of chemicals on humans.

4. What federal agencies are likely to be pace setters for developing advances in toxicological testing technology?

New information and experimental techniques which may lead to new approaches or refinements in toxicological testing more likely will come from the laboratories of such research groups as the National Institute of Environmental Health Sciences and the National Center for Toxicological Research. Although regulatory agencies will continue to require new toxicological data on specific chemicals, they cannot be expected to be leaders in developing new standards, since their prime responsibility is that of regulation, not the development of new information.

It would appear that the federal research laboratories who engage in basic research will be the most likely centers for advances in toxicological testing methodology.

5. What will be the impact of mutagenic screening on overall toxicological testing requirements in the future?

It is expected that mutagenicity testing will continue to increase in importance as a useful tool for assessing the toxicological hazard of candidate test materials.

In the November 13, 1985 Federal Register, the Environmental Protection Agency published "Proposed Guidelines for Mutagenicity Risk Assessment." The fact that these proposed guidelines, which have been under development for over two years, have reached the public is a meaningful implication for the future role of mutagenicity testing. The importance EPA places on mutagenicity information for future regulatory activities is shown by the following, taken from the referenced document: "The EPA will utilize procedures described herein to evaluate the risk associated with the exposure of humans to chemical mutagens. The mutagenicity risk assessments prepared pursuant to these guidelines will be utilized within the requirements and constraints of the applicable statutes to arrive at regulatory decisions concerning mutagenicity."

Although the Food and Drug Administration has not taken a public position on the need for mutagenicity information on candidate products that come under the FDA's purview, it is well known that the clearance of the material by FDA will be enhanced if mutagenicity data on the chemical under consideration is available. Only this week, a FDA staffer told this commentator that, for the foreseeable future, FDA will not require the inclusion of mutagenicity data in a submission which includes an evaluation of the hazard of the material. They do recognize, however, the value of these kinds of information as guides toward selection of the mammalian studies that eventually will be done on a candidate test material.

Currently, there is close coordination between the EPA and the European OECD in developing mutually acceptable toxicological testing protocols. Within the next month, procedures for acute toxicological studies which have been agreed upon by OECD and EPA will be published in the Federal Register. Also, OECD has issued proposed guidelines for mutagenicity testing, and the EPA has this document under advisement. It is expected that the OECD approach, or a closely related plan, eventually will be accepted by the Environmental Protection Agency.

6. Will the concerns for possible synergistic effects due to multiple chemical exposure significantly impact on short- or long-term toxicological testing requirements?

   The concern of possible synergism or potentiation between two or more chemicals is a problem that has had repeated attention for many years. Although there have been many attempts to develop principles for predicting interactions and resultant synergistic responses, nothing of significance has endured which provides guidance on this subject. This issue was recently addressed by the National Academy of Sciences and the resultant report essentially confirmed the previous statement. The lack of basic information on this problem does not minimize the concerns and needs. Today, the question of potential synergism between compounds can be answered only on an issue-by-issue basis, as such arises.

7. Possible future role of structure activity relationships to toxicity testing requirements.

   Today there are various groups active in developing systems which expand the bases of information about structure activity correlates, in relation to responses. The field has advanced so that now a number of computerized data bases are available (such as QSAR and PROPHET) which allow a user to make theoretical molecular changes in structure and then obtain theoretical toxicological data related to the structural change. A three-day meeting on this subject area will be held at Raleigh, NC, in February, 1981. The purpose of this industry/government sponsored symposium will be to develop further the tools which may be used in setting priorities for toxicological testing of chemicals, as well as to foster discussions and collaborative interactions of investigators in this area of research.

   It is forecasted that the use of structure activity information will not so much eliminate certain toxicity testing requirements, but rather, will allow more effective and efficient planning of toxicological procedures before they are undertaken.

8. Will neurotoxicity and behavioral effects testing become a part of toxicological regulatory requirements in the near future?

   There are many who are interested in utilizing neurotoxicological or behavioral techniques to evaluate compounds in greater depth than has been possible in the past. There is potential for these new systems in future regulatory decision making. Both disciplines, however, have constraints which will make their acceptance a slow process. The sophisticated *in vitro* neurological evaluation systems, as developed by Spencer and Schaumburg, currently have a limited number of scientists capable of conducting these techniques. On the other hand, the sensitivity of these systems undoubtedly will attract many investigators over time. Although there is a wide-spread interest in the area of behavioral toxicology, attempts to evaluate such responses in toxicological evaluations suffers from a lack of specificity. There is need to develop a behavioral data base which can be quantified so that there is wide-spread acceptance in applying the resultant test information to man. At present, that kind of a base does not exist, but it can be expected to develop as the field matures.

It is believed that neurotoxicological and behavioral testing eventually will become an accepted part of regulatory decision making, but only on a gradual basis over extended periods.

9. What impact will the concern for safety and health of persons performing toxicological testing have on future testing research requirements in this field?

   Already there is recognition of the potential health hazard to laboratory staff from various chemicals when tested for toxicological significance. In the last few years, increased precautions have been taken to protect the laboratory workers through improvements in working conditions and facilities (such as improved ventilation systems, barrier controls, protective clothing, and the like). The cost for such improvements will, in the long run, be borne by those who pay for the investigations. Thus, these types of increased costs, when conducting future toxicological studies, may impact on the total numbers of studies that may be conducted--inasmuch as there always are finite amounts of funds for such work.

10. What advances are likely in non-animal testing that will reduce the amount/extent of animal testing?

    The use of short-term, rapid screening systems undoubtedly will find increased application as their data bases develop to show the reliability and correlation of in vitro test data to that of the in vivo tests. In addition, non-mammalian systems, such as the use of Hydra for screening potential teratological effects of chemicals, appear to have an interesting future. Likewise, the in vitro neurotoxicological systems of Spencer et al. has already been discussed.

    Thus, it would appear that in vitro systems and short-term, less expensive, non-animal test systems will find increasing use as predictors of possible toxicological responses; such information undoubtedly will require confirmation in whole mammalian systems.

TR-477-14-4

FORECAST OF POTENTIAL TECHNOLOGY CHANGES THAT MAY IMPACT TOXICOLOGICAL TESTING REQUIREMENTS

ICAIR Task Assignment No.: 107
Task Assignment Title: Impact of Changes

Task Assignment Report

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IMPACT OF CHANGES

In^ oduction

The task assigned focuses on toxicological testing. The forecast of changes in epidemiologic research does not fit easily into the same outline as the other aspects of the task. Consequently, I have taken some liberty in addressing the issues from an epidemiologic perspective.

The purpose of toxicological testing is to assess the potential hazard to human health for the agents tested, so that these hazards can be reduced or avoided. A primary intent of such testing, though not always achieved, is to use the screening process to identify the problem before any material human disease occurs. Epidemiology, on the other hand, can only bring to light hazards which have already caused human disease. Though epidemiologic research is considered by many health scientists as the cornerstone of preventive medicine, epidemiology is fundamentally a reactive discipline, restricted to studying the effects of health hazards which have already had an adverse impact. This reactive nature of the discipline is an inevitable concomitant of the focus on human disease.

A reactive response may seem lamentable, but in many instances, no alternative exists. Furthermore, epidemiologic information does permit, without extrapolation across species, relatively good estimation of actual human risks and the amount of disease that intervention prevents. The delay before preventive measures are possible is inevitably longer for diseases which have a long induction time between the action of a toxic agent and the appearance of disease. For example, cigarette smoking became popular in the United States abut the time of World War I. Nevertheless, it was decades before the lung cancer epidemic which ensued was noticed by public health researchers, and even longer before this epidemic was linked to cigarette smoking. Even if cigarettes had been banned in 1964, when the highly publicized Surgeon General's (1964) report on smoking was released, the incidence rate of lung cancer would have continued to climb for many years before the epidemic peaked. The lengthy induction time between the effect of cigarette smoke on the bronchiolar epithelium and the subsequent development of lung cancer guaranteed that the epidemic of lung cancer would have continued to wax long after any effective preventive measures were instituted. It is clear that toxicologic testing on animals could have detected such a health hazard decades before it became evident in humans (though our recent experience suggests that the results of such testing have had surprisingly little impact).

The preceding problem, although it is inherent in epidemiologic research, can be mitigated. Variation in induction times usually results in some cases of disease which are causally related to a toxic agent appearing relatively soon after the time of action of the agent. These cases may occur primarily among those with extremely high exposures to the toxic agent. Such early effects can be detected by directed surveillance activity; the necessary feature of such surveillance is that the size of the population studies must be large, the exact size depending on the magnitude of the effect. The great expense of large, directed surveillance activities makes it unlikely that many hazards will be detected earlier than on a purely reactive basis using such an approach, unless changes in epidemiologic research occur during the years

ahead. The changes which are likely to occur will be in the areas of data resources and epidemiologic analysis.

Data Resources

The major innovation affecting availability of data is, in all likelihood, the National Death Index, which was begun in 1979 by the National Center for Health Statistics. This index will facilitate the identification of deaths among population subgroups of particular research interest. Because the Index was started only in 1979, relatively few deaths have accumulated, and as yet the Index is not a valuable epidemiologic resource. With the passage of time, however, the number of deaths available for study will gradually increase, as will the range of time over which secular trends may be measured, gradually increasing the value of the index. (Retroactive indexing, though feasible in principle, is expensive and is therefore unlikely to be attempted in the near future.)

Social Security records contain useful information on employment and vital status, offering great potential for epidemiologic research that has not been realized. The increasing use of the Social Security number as a unique number for record linkage and identification for many purposes adds to the potential. For this potential to be realized, however, would require a reversal of the trend toward restriction of record linkage and restriction of access to records to safeguard individual privacy. Recent failures to pass legislation exempting epidemiologic research from certain privacy restrictions indicate that prospects are currently dim for any changes in public policy in the direction of increasing either record linkage or access of epidemiologists to Social Security or related data. In a recent paper arguing for more liberal access to such valuable epidemiologic information, MacMahon (1979) predicted that "an enlightened attitude towards the use of personal records for research should lead to a far greater proportion of our experience being used to provide information of practical value to our own and future generations." MacMahon offered no indication, however, that such an attitude would soon emerge in this country.

Epidemiologic Analysis

Advances in epidemiologic analysis will not influence the assessment of toxicologic hazards substantially unless such advances involve new modes of analysis which have striking advantages of validity or precision over the methods currently available. Some gain in the broadest sense of scientific efficiency should be expected, however, as the rapid theoretical innovation of the past 10-15 years gradually becomes adopted by the epidemiologic community. For example, multivariate analysis of case-control studies with individual matching and the proportional hazards analysis of Cox are two recent methodologic advances which are only beginning to be used routinely by those conducting epidemiologic research. Wider adoption of these techniques and others already published should lead to slightly more efficient studies and a shorter period from initial publication on a specific association to the point of general consensus by many scientists. Consensus should come more quickly because inefficiency in analysis is a major source of potential disagreement among several studies of the same question.

A manuscript of mine addresses the question of inefficiency in analysis introduced by implicit assumptions about induction time that are incorrect. Such incorrect assumptions are equivalent to misclassification errors, or tend to mask the presence of real effects. I believe that such inherent errors in epidemiologic research decrease study efficiency substantially and contribute heavily to the delay in consensus on specific public health associations. My paper offers a prescription for this problem, namely, the explicit fitting of various induction-time assumptions in each analysis. The suggested procedure will eliminate the misclassification error from faulty assumptions about induction period and simultaneously provide information about the length of induction period. If this analytic technique were widely adopted, I believe it would lead to a substantial increase in the efficiency of epidemiologic analysis, by reducing a large source of error built into epidemiologic studies.

Conclusions

Advances in data resources and epidemiologic analysis seem likely to produce epidemiologic detection or confirmation of toxicologic hazards more rapidly than occurs at present. This prediction must be tempered, however, by a simultaneous consideration of the totality of epidemiologic resources available for such activities.

Maclure and MacMahon (1978) concluded that evidence linking environmental agents to human cancer has been accruing in an exponentially increasing manner (see figure). It would be impossible for such exponential growth in knowledge of environmental agents to continue indefinitely; neither the availability of scientific resources nor the number of agents available to evaluate will permit prolonged exponential growth of this curve. During the recent period demonstrating exponential growth, there has also been rapid growth of funds directed to epidemiologic research and training. More recently, such funds have leveled off, and perhaps declined in real terms. Even with more information data resources and more efficient epidemiologic analyses, it is likely that the rate of growth of epidemiologic knowledge about environmental factors will decline. Nevertheless, in absolute terms, we can surely expect that knowledge linking environmental agents with human disease will accrue faster in the years to come than it does today.

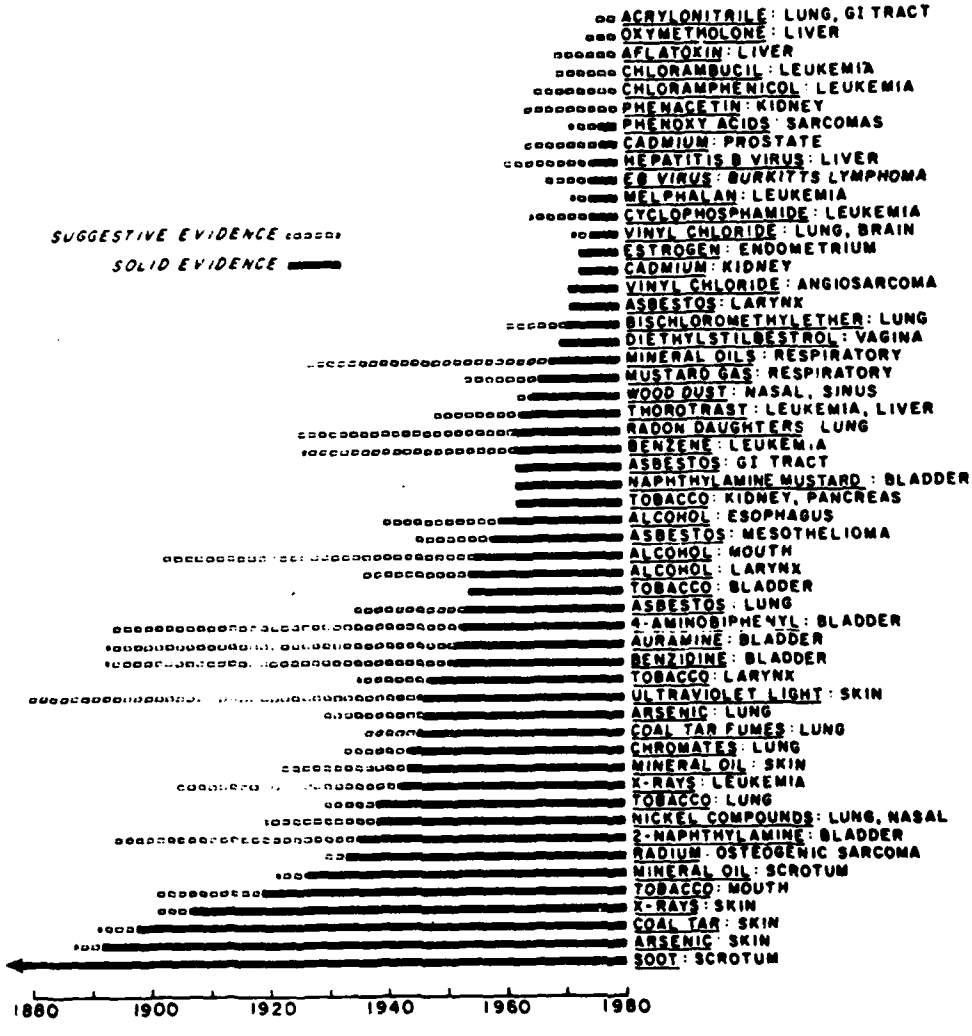

FIGURE 1 ENVIRONMENTAL CANCERS:

HISTORICAL DEVELOPMENT OF EVIDENCE IN HUMANS

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TR-477-14-5

FORECAST OF POTENTIAL TECHNOLOGY CHANGES THAT MAY IMPACT TOXICOLOGICAL TESTING REQUIREMENTS

ICAIR Task Assignment No.: 107
Task Assignment Title: Impact of Changes

Task Assignment Report

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IMPACT OF CHANGES

1. Forecast the level of support for toxicology research and development, especially in the areas of basic research and its impact on technology advancement.

   Two opposing forces are likely to dictate support for toxicology research. Implementation of the policies of the new Administration will result in a reduction in funds in areas related to the environment and toxicology. On the other hand, the increasing number of lawsuits involving toxic torts will force industry to examine the cost effectiveness of selling products without rigorous examination for possible toxic effects vs. that of conducting thorough toxicology tests prior to marketing. The overall effect of these two trends will probably result in the same rate of increase of funds for toxicology research over the next years as that experienced in the previous five. Technology advancements will therefore appear at a continuously accelerated rate. Industry and Federal agencies will increasingly fund universities for basic research in toxicology.

2. Are advances/standardization of neurotoxicity and behavioral effects testing believed likely to take place in the relatively near future so that these types of effects will have greater acceptance as a basis for establishing rules and regulations?

   Significant advances in neurotoxicity occurred during the 1970's and these have resulted in a series of approaches to assay compounds for chronic neurological effects. The best validated approach is examination of selected areas of the nervous system (brain, spinal cord, peripheral nerves) for pathological changes using contemporary morphological methods. These methods, originally designed for electron microscopy of tissues, involve optimal fixation of tissues with buffered glutaraldehyde, stepwise dehydration, embedding in epoxy resin, preparation of one-micrometer thick sections stained with toluidine blue, and examination with the light microscope. Such preparations offer vastly superior detail and resolution compared with the conventional histological technique which employs paraffin sections stained with hematoxylin and easin, and other histochemical stains. Neuropathological changes are much more readily observed and accurately described.

   Other approaches involving evaluation of whole animals include: (a) behavioral testing -- not yet validated, (b) tests of nerve conduction (promising but restricted to chemicals acting on the peripheral nervous system), and neurological examination (not validated for small animals and unlikely to be as sensitive as contemporary neuropathological methods).

3. What advances are likely in non-animal testing that will reduce the amount/extent of animal testing?

   Tissue culture methods to assay chemicals for neurotoxic properties are being developed rapidly. The development of one method -- organotypic tissue cultures composed of structurally and functional coupled explants of spinal cord, dorsal root ganglia and striated muscle -- is already well advanced. This testbed has been validated for several classes of neuro-

toxic chemicals but still requires substantial further development and simplification. The TSCA Interagency Committee has recently recommended funding of this system to OMB.

Other tissue culture methods (reconstituted and dissociated systems) have not been validated but progress may be rapid in this area.

4. Forecast equipment needs for laboratory neurotoxicology testing.

State-of-the-art neuropathological assessment with the light microscope has relied on expensive equipment (e.g., ultramicrotomes) designed for the more stringent requirements of electron microscopy. These techniques also limit the area of tissue examined, a major disadvantage for the assessment of brain pathology. Equipment manufacturers have very recently responded to these need by marketing heavy-duty microtomes suitable for the preparation of large areas of 1-2-micrometer thick epoxy sections. Such microtomes utilize stainless steel knives (suitable only for certain plastics) or wide glass knives prepared by special knifebreakers. Automatic tissue processors are available for chemical preparation (fixed and dehydration) of large amount of tissue but these are not yet reliable. Optimal preparation of tissue is achieved by intracardiac perfusion of animals with fixatives delivered with the aid of a perfusion pump. Histological slides are examined with a binocular bright-field light microscope (double-headed for simultaneous, dual observation). Peripheral nerve fibers can also be usefully examined by teasing apart a nerve bundle with the aid of mounted sewing needles and a stereoscopic, variable magnification dissecting microscope.

Tissue culture methods require sterile hoods (often in purpose-built rooms with positive-pressure ventilatin), an ultra-pure water supply and light microscopes provided with special objective lenses with long working distances.

5. What will be the projected speed of acceptance of technology changes for toxicology testing in neurotoxicology?

Industry will rapidly (within 10 years) switch from conventional techniques of neuropathology to contemporary methods of analysis provided that the latter is cost effective. The newer techniques are presently more expensive but, with increased demand, equipment and consumable costs could be reduced. Some retraining of technical and professional personnel will also be required.

Industry will continue to experiment with simple, inexpensive behavioral tests,but these are unlikely to provide an effective screen for neurotoxicity and can never replace histological examination of nervous system. Tissue culture methods will be introduced more slowly (over 20 years) if reliability and reproducibility can continue to be demonstrated over the next five years.

6. What is the anticipated pace at which these screening techniques are likely to receive full acceptance as the basis of regulatory actions?

1. Contemporary histological methods of nervous system examination for pathological changes.....3-10 years.

2. Behavioral screening techniques are of great interest to FDA and EPA largely because their professional staff in neurotoxicology has a background in psychology and behavioral toxicology. Such techniques may be accepted prematurely (within 5 years) and later rejected as inadequate.

3. Tissue culture methods to screen for neurotoxic chemicals.....20 years.

4. Neurochemical methods providing rapid, automated screening of large numbers of chemicals may become a possibility by 2020. This approach will provide a first-pass method of detecting neurotoxic compounds, singling out individual agents which can then be evaluated by tissue culture or whole-animal techniques. Such an advance would lead to a dramatic decrease in the numbers of animals required for toxicity screening.

7. What technology changes are anticipated in the area of animals used for neurotoxicology testing?

   With the exception of the chicken assay for organophosphorus neurotoxicity, the rat will continue to be the principal choice. Two-species testing is likely to become fashionable and the mouse is the likely second animal. Special focus will have to be placed on normal changes accompanying age in order to distinguish neurotoxic damage in animals tested for lifetime exposure. Use of primates will expand for the validation of neurophysiological and psychological tests designed to monitor humans for subclinical neurotoxicity.

8. What technology changes are anticipated in the area of duration of neurotoxicology testing studies?

   Studies of acute effects will be used more for range-finding purposes for subchronic and chronic studies than for purposes of establishing the neurotoxicity of a compound. Much greater attention will be given to subchronic (90-day) studies in this decade. Chronic (lifetime) studies will also increase as a result of heightened concern over the effects of human lifetime exposure to toxic substances and the possible special vulnerability of individuals in old age.

9. Will it be possible to use non-inhalation toxicology data to predict human health hazards associated with inhalation exposure?

   Breakthroughs are anticipated in methods to predict human health hazards from animal exposure. These will involve measurement of relative absorption (e.g., respiratory), relative metabolism and relative excretion between two experimental species and man. These data, coupled with information on the differential neurotoxic response of two experimental species to repetitive exposure, will allow reasonably accurate prediction of acceptable human exposure. As a result of this anticipated technological breakthrough, and because of the high cost of inhalation toxicology, use

of the latter in predicting human health hazards is likely to become more restricted.

10. Forecast potential advances in data evaluation and interpretation techniques that may permit improved communication of the results of toxicity testing to both decision makers and the general public.

    The present trend is disappointing. Federal pressures on industry and individuals to report toxicology test data rapidly are causing reports to be published prior to the usual and accepted method of peer review by a respected scientific publication. If this disturbing trend continues, toxicologists will experience an even lower credibility among the public than they presently enjoy. Data evaluation and interpretation per se will improve markedly with the introduction of new techniques of tissue evaluation (see 2 above) and wider dissemination of neurotoxicology information to testing laboratories. As information accrues on large numbers of chemicals, the relative toxicities of each will become more apparent. As a result, some compounds once considered potent toxic agents may be subject to reevaluation of their toxic properties relative to those of other newly examined compounds.

11. Under the assumption that toxicological testing requirements will become more stringent, what will be the future value of neurotoxicological data developed under present day standards?

    Neurotoxicology research is presently engaged in the task of reevaluating the vast majority of inadequate neurotoxicology data that have emanated from toxicology testing laboratories during the last 30 years. Provided this task is achieved with rigorously controlled exposure methods, positive and negative controls, animals of both sexes, 2-3 exposure levels for periods up to 90 days, and contemporary techniques of morphological examination, the data will be extremely reliable for future standards. Behavioral toxicology and teratology will continue to flag compounds with acute neuroactive (neuropharmacological) properties, but these may not represent a toxic threat following low-level, long-term exposure.

12. What will be the future impact/role of epidemiology studies in human health hazard assessments?

    Such studies will continue to identify new neurotoxicological health hazards and play a major role in the identification of causative agents in neurotoxic outbreaks.

13. Forecast the role of structure-activity relationships as they may replace certain neurotoxicity testing requirements.

    With the exception of the organophosphorus pesticides, ability to predict neurotoxicity from examination of chemical structure is poor. Positive developments are anticipated in this area but the problem of dealing with so many different chemical groups is so vast that it will have little impact as a substitute for neurotoxicity testing in the next 20 years. Advances in this area will depend on a substantial amount of basic research on mechanisms of neurotoxicity at the molecular level, an approach which is presently in its infancy.

14. What will be the influence of current basic research investments on the future testing technology?

Current basic research will have a major impact on future testing technology in neurotoxicology. Breakthroughs in the classification of neurotoxic disease according to cellular target site have been made recently. This provides a cogent basis for (a) selection of sites for neuropathological examination to detect very early changes and (b) the design of studies (behavioral, neurological, electrophysiological) to detect these cellular changes. Major future investment in basic research in tissue culture and neurochemical approaches will enable technology advances that will simplify and speed toxicology screening using automated, in vitro techniques.

15. Forecast the availability of scarce personnel for neurotoxicology research and testing.

Unless there is a major influx of industry and federal funds into universities, there will be a desperate shortage of trained professional and technical staff. For neurotoxicology assessment, pathologists and veterinary pathologists require retaining in neuropathology and contemporary morphological techniques. Histological technicians require further training. Individuals trained in neurophysiological and tissue culture techniques are also in short supply. Behavioral toxicologists are more abundant but most lack training in neuroscience, neuropathology, neurophysiology and neurochemistry. Neuroscientists are plentiful: few have been attracted into neurotoxicology, but the number will increase rapidly as funds for other areas of basic neuroscience become scarce. Ph.D.'s and D.V.M.'s, rather than M.D.'s will play the major role in neurotoxicology research and toxicity evaluation (including pathology).

16. Will concern for synergistic/potentiating effects due to exposure to multiple chemicals significantly impact short- and long-term neurotoxicological testing requirements?

This is a major concern in the petrochemical industry at the present time. They have decided that costs for whole-animal studies to examine such phenomena are prohibitive and, as a substitute, are beginning to exploit the potential of the organotypic tissue culture system described above. Potentiation of the neurotoxic effects of one chemical by a second non-neurotoxic agent has been demonstrated with this system. Synergistic studies have yet to be conducted.

17. Forecast the degree of concern for safety and health of persons performing neurotoxicity testing and what impact this will have on future testing resource requirements.

A moderate level of concern will be maintained but enactment of OSHA-type safety requirements will be rigorously opposed (and defeated) by organizations representing universities. Industry will continue to build sophisticated facilities to protect the health of toxicology testing personnel. Incentives may be given by the federal government and industry to

allow universities to comply with GLP regulations and thereby accept contract work.

18. What technology changes are anticipated in the areas of routes of exposure for neurotoxicology testing?

    There will be a much greater emphasis on the role of percutaneous absorption in producing neurotoxic effects. Focus will be placed on relative absorption from different routes of exposure and between species. This, coupled with studies on relative metabolism and excretion between individuals and species, is likely to provide explanation for differential individual and species susceptibility to neurotoxic agents, and provide a coherent base for the prediction of human health hazards.

19. Evaluate the role of scientific/professional societies in promoting technology changes that will impact testing requirements.

    Professional toxicology societies have been, and will continue to be, concerned about professional standards of conduct in the science of toxicology. Moves already taken will raise the standard of training as accreditation in toxicology becomes more widely accepted. Societies will also display a small role in promoting basic science toxicology research in universities by providing a mechanism for the distribution of scholarships awarded by industry.

20. What impact will medical treatment/advances have on reducing concern with certain adverse toxic effects?

    Basic science research is presently generating some novel techniques for the assessment of neurotoxicity in man. The implementation and development of these screening techniques will allow detection of subclinical neurotoxicity and precipitate removal from the toxic environment and identification of the causative agent. Neurologists will continue to utilize advanced equipment (e.g., computer-assisted tomography, somatosensory evoked potential systems, electromyography) for the detection and assessment of neurotoxic damage in man.

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FORECAST OF POTENTIAL TECHNOLOGY CHANGES THAT MAY IMPACT TOXICOLOGICAL TESTING REQUIREMENTS

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Task Assignment Title: Impact of Changes

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IMPACT OF CHANGES

Interpretation of Toxicity and Assessment of Risks to Human Health

1. As criteria evolve for the evaluation of the adequacy of toxicologic data, what impact might be expected on the characterization of previously completed toxicologic work?

   This issue has been, and will continue to be, a perennial problem. Interest in the problem has been heightened predominantly by activities of EPA (principally the internally generated re-evaluation of herbicides) and of FDA (particularly the congressionally stimulated institution of the "cyclic review" of GRAS compounds and continuing review of food colors). The question demonstrates the frequent inconsistency between legal requirements and scientific developments. When statutes and regulations were instituted 10 or 20 years ago, there was concern--albeit weak--of "freezing" science at one particular time. Yet there was a need to arrive at legally defensible and scientifically sound conclusions at the time of the regulatory action. The acceleration of testing methodologies and of data interpretation philosophy has now created a chasm between existing criteria and those used only 5 or 10 years ago. In view of anticipated progress in risk assessment methodologies and in the development of a wider array of testing modes and systems, the problem will continue at a quickening pace. There are two possible types of solutions: first is the grandfathering of risk assessment decisions thereby removing from future consideration all subsequent reviews of previously acceptable information; the second is a cyclic review of toxicity studies and results based on some form priority system. It is unlikely that the first option would be accepted generally because of the pitfalls derived from the generation of new data (e.g., human studies) and from the likely loss of credibility (is it "safe" or not?) The second option offers a more intellectually satisfying solution but one that has many long-term and far-reaching impacts on society. Such impacts include (a) the further restriction of existing resources (both laboratories and manpower) from work on new materials (thus, restricting economic and welfare progress), (b) the cost of repeating experimentation, and (c) the burden to government in simply reviewing and evaluating the new data and reconciling conflicting information. For those considerations, broad scale (i.e., many classes) cyclic retesting and re-evaluation must be restricted to a comparatively small number of compounds. As confidence increases in making priority selection judgments and as resources are used more efficiently, the cyclic progress will gain momentum; however, level of equilibrium, while undoubtedly finite, is as yet unclear.

2 At what pace is risk assessment technology progressing?

   Risk assessment broadly defined encompasses the intellectual analysis of information to describe risks and the probability of their occurrence. In a restricted sense, risk assessment describes the extrapolation of laboratory data (qualitative and quantitative) to humans. Within that context, the concepts used to undertake this process are undergoing extensive scrutiny and consequent evolution. For example, in the qualitative extrapolation process (e.g., predicting teratogenicity in humans from teratogenic observations in other species) is now taking greater cognizance of

substantial differences in xenobiotic metabolism, in physiologic parameters (e.g., plasma proteins), and in repair mechanisms. It is now recognized that rodents are imcompletely predictive of target organ toxicity, that there are great differences in potency among species, and that individual susceptability within exposed numbers of a species can be quite varied. Several scientists are engaged in this development of techniques to amalgamate these factors to perform qualitative risk estimates. It can be anticipated that in the near future the process will become complex intellectually and that the recognition of the diversity of elements will lead to the requirement for more extensive information from laboratory studies. Other possible consequences are the use of animal models that were previously considered unconventional (e.g., primates) and the development of in vitro systems using human cells for increased confidence in the species cross-over. Cell cultures will have added advantages of easier manipulation, lower cost, and less time to develop data on adverse effects, mechanisms, and metabolism for comparison with those from surrogate species.

Qualititative (or dose-reponse) extrapolation has progressed considerably in the past few years despite considerable controversy. The advances have been mainly statistical (e.g., greater understanding of changing confidence intervals with lower dose extrapolation) and very little biological. As a result of the in-depth scrutiny, some concepts may be greatly modified in the next decade. One example is the no-adverse-response level (NOEL). There is increasing recognition that most laboratory studies yield a no-adverse-response level and that that value is restricted by design and extrapolation considerations. The NOEL is an operational threshold for a specific circumstance and does not necessarily describe the threshold in another population. This does not deny the existence of biologic thresholds, but merely acknowledges our present inability to extrapolate thresholds in rodent studies to thresholds in larger and more diverse population of humans. In addition, it does not describe how to measure the true biologic threshold. The impact is likely to be, first, the use of quantitative expressions of risk for all toxic manifestations and, second, the encouragement of more innovative and extensive research on the toxic properties of substances (for example, pyramid designs, more specialized biochemical and genetic studies, and more emphasis on rates of effects and reversals). It will also offer an opportunity for cost-benefit analysis: if conventional experiments are required, a pre-set risk level could guide interpretative judgments, but broader experimental opportunities would exist to increase confidence and refine risk estimates if the potential benefits outweight the costs. Generic experimentation conducted perhaps by NIEHS, NCTR, and CIIT are likely to open the door to such advances by 1990.

3. To what extent will reversability and irreversability of toxic lesions influence the evaluation of toxic risks?

There is little doubt that this discrimination will continue to be made. However, there are likely to be at least two fallouts: first, there will be increased insistence to document, rather than assume, reversability implying the need for additional experimentation; second, there will be substantial impetus to assess the rate of reversal in both the test species and in humans to improve confidence in extrapolation implying the

need for extensive basic reasearch on various types of toxic responses at varying levels of biologic organization.

4. To what extent will communication of complex scientific information and concepts to non-scientists change? In which direction?

   The past decade has demonstrated a decreasing ability to effectively communicate scientific findings and concepts to non-technically trained individuals. The result has been greated reduced credibility of scientists. Some response to the problem has been forthcoming from many quarters. The news media, for example, has been forming many different educational programs in the sciences for the benefit of media reporters. Consumer oriented organizations, such as Consumers Union, has expanded greatly its efforts to educate its readership about complex scientific issues surrounding toxicologic questions such as the nitrite controversy. And the National Academy of Science has been seeking improved methods of transmitting the scientific content of its reports to the lay public. It is unclear what impact such efforts will have because the outcome may depend in great measure on unpredictable factors.

Toxicity Testing Methodology

1. Will the expressed need to obtain toxicity information about mixtures lead to changes in experimental protocols and requirements?

   The proper testing of complex mixtures still faces many technical difficulties. However, much research is being conducted on the basic understanding to toxic interactions (by NIEHS and NCTR) and on the testing of mixtures (by EPA). Consequently, many of the technological difficulties are expected to be resolved over the course of this decade such that the reliable testing of mixtures will become reasonably prevalent in the near future. There will be compounded effects from such experimentation: primarily the additional testing of fractions of individual components. This will be a necessary step where engineering technology will be required to alter processes to reduce or eliminate more hazardous and less desirable parts of those processes.

2. How can knowledge of structure-activity relationships (SAR) play a role in toxicologic testing?

   Interest in SAR has gained momentum because of the hope that it could replace, at times, the need for laboratory studies. The opposite now appears to be emerging as the result of proposals by the Office of Pesticides and Toxic Substances of EPA. Thus, the use of SAR to prioritize compounds for specific types of tests (e.g., reproduction or cancer) appears to be the most generally acceptable use by the scientific community. Because of the many exceptions to SAR hypotheses, it is unlikely that SAR will be used in lieu of laboratory studies. However, by suggesting the research path, SAR may lead to the need for fewer, perhaps more complex, and hopefully, more relevant studies to serve as a basis for risk analysis.

3. For predictive toxicity studies, what routes of exposure should be utilized?

For many decades, an axiom guiding predictive toxicity studies has noted that studies in animals must use the same route of exposure as that known or anticipated in humans. In the planning of studies, it is doubtful that this will ever change, even for the inhalation route. Because of the various uncertainties in species extrapolation, this one should be maintained as much as possible. However, in the interpretation of existing data, it is recognized that information from studies using the other-than-described routes may be all that is available. In the past, such situations have been addressed by the application of relatively simple assumptions and calculation. In the future, such situations are likely to require highly extensive toxicokinetic information to make the appropriate crossover from one route to another. This will be necessary because of the increasing recognition of the extensive complexity associated between route of exposure and effective dosage at the target tissue level.

TR-477-14-7

FORECAST OF POTENTIAL TECHNOLOGY CHANGES THAT MAY IMPACT TOXICOLOGICAL TESTING REQUIREMENTS

ICAIR Task Assignment No.: 107
Task Assignment Title: Impact of Changes

Task Assignment Report

by

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IMPACT OF CHANGES

This evaluation will focus specifically on the role of the use of structure-activity relationships in the future for toxicological testing standards and protocols. This approach has been advocated by me over a period of years as part of the tier system for toxicological evaluation; in part it constitutes the first stage of such a tier system. A reprint (reference 1) indicates the value of this tool. However, it should be pointed out that the utility of this parameter rested, in my work, on the availability of a variety of biological tests. The latter included specifically: mutagenicity testing, transformation in cell culture and carcinogenicity testing in vivo. Furthermore my own work is based on restricted compound types. These types are: (1) alkylating agents; (2) acylating agents; (3) chloro-ethers and (4) halogenated hydrocarbons.

Compound types (i) to (iii) include direct-acting agents, i.e., those that do not have to be metabolized in order to exert their biological activity. They are therefore positive, when biologically active, in the three bioassays mentioned above, be it in vivo or in vitro. The halogenated hydrocarbons are not always direct-acting agents. Most of them belong to the large group of carcinogens which I refer to as indirect-agents. These agents have to be metabolically activated in the animal or in cell culture to an activated intermediate. In the case of mutagenesis bioassays in mammalian systems these chemicals are "activated" for assay in vitro by rat liver microsomes.

Indirect-acting agents, and I will address myself largely to carcinogenic compounds, consist of a wide variety of compound types. The compound types which have been tested extensively in various species of animals and routes of administration are the following: armoatic hydrocarbons, aromatic amines, nitroso compounds, halogenated aliphatic and olifinic hydrocarbons, aflatoxins and others.

A vast literature is in existence on bioassays for some of these compound types. Sporadic and/or concentrated efforts have been made with some of these to systematically compare chemical stucture and carcinogenic or other biological properties. In my view we have only scratched the surface in attempts at using the bioassay data in order to learn prediction of carcinogenicity of untested compounds in the various classes enumerated above. It is with these compound groups and to a lesser exent, direct-acting agents, that concentrated efforts are needed. The use of computerized methods might be seen as a practical approach in the future for such studies.

Apart from direct examination, studies on the reactivity of direct-acting agents can theoretically be readily carried out. Such reactivity studies should consist of their reactions with nucleophilic reagents (i.e., molecules containing, for example, amino-, sulfhydryl- and hydroxyl groups). Along with reactivity studies, other factors which need to be considered and that can possibly be computerized are; molecular functionality (i.e., monofunctional, bifunctional, etc.), molecular flexibility (i.e., rigid or flexible) and stenochemistry (i.e., availability of reactive sites or crowding of reactive sites by bulky substituent groups). Some of these factors are described and examples of them are given in reference 1.

In the case of direct-acting carcinogens the situation is much more complicated. This is so because the activated carcinogenic intermediates are known in only a few of these compounds (see references 2, 3, 4). In most cases this situation prevails because these intermediates are formed in situ and react at the target site very rapidly. I propose that better analytical techniques be developed in order to trap activated intermediates at their site of reaction, and to isolate the product(s) such intermediates form with reactive nucleophilic sites on biomacromolecules. This will greatly aid in structure-activity studies.

A number of approaches need consideration and development as support studies for structure-activity and toxicologic evaluation.

(i) A consideration of metabolic pathways (known and/or suspected) by simply writing down the various possibilities. This has been done (references 5 and 6), and this simple approach has proved successful in the halogenated hydrocarbon series (reference 1). A far more sophisticated method has also been suggested by the use of computerized methods. This latter method is in its infancy and will need collaboration between computer experts, chemists and toxicologists (reference 7).

(ii) Assuming that reactions with DNA or the chromatin complex of the cell are the critical reaction sites where carcinogens exert their genotoxic effects, much more needs to be known about these reactions and the consequences of these reactions. This statement is based on the knowledge that very minute changes in chemical structure of potent carcinogens, e.g., addition of one or more methylene groups, can and do result in greatly diminished or loss of carcinogen activity. Again, we are dealing with an area in which sporadic efforts have been made, but really concerted efforts have not been undertaken. I state this, not to denigrate the many carcinogenesis researchers who have worked in this important area, but to point out the importance of a concerted effort.

(iii) Closely related to point (ii) above is the role of repair enzymes of DNA and the effect of the structure and site of reaction of a chemical on DNA. Thus, many chemicals will bind covalently to DNA and result in isolable products. Nevertheless, these chemicals are not carcinogenic, presumably because DNA repair enzymes have effectively removed those sections of the DNA helix affected by the chemical. On the other hand, some very potent carcinogens, i.e., bis (chloromethyl) ether show an extremely minute extent of binding to DNA; in fact, the portions of DNA affected by this carcinogen have yet to be isolated (Van Duuren unpublished data; see also reference 1).

Apart from carcinogens there is a whole group of compunds of various structures which are referred to as tumor promoters and carcinogens. These are epigenetic factors (as opposed to genotoxic carcinogens) which profoundly alter the effect of a carcinogen. We are only beginning to learn about structure-activity and possible modes of action of these compounds (reference 8). This is an area where a great deal of effort is needed; it is also an area where the regulatory agents are completely at a loss. Many of these agents are ubiquitous in our environment and hence the concern of regulatory agencies.

In past years we have, like others, propounded the tier system for carcinogen evaluation. The tiers were, in order of increasing importance: (a) structure-activity; <(b) short-term assays; <(c) animal bioassays, <(d) epidemiology. This tier system is now regarded as almost obsolete, because of the cost and time involved in (c) and (d), and the urgency of the regulatory agencies to rapidly arrive at rationale decisions. It has, for example, become clear to the agencies, to industry and to academia that carcinogenicity data are in many cases incomplete or unreliable and that epidemiology is frequently non-existent. The roles of (a) and (b) in this paragraph have therefore taken on added importance. Implementation of concerted efforts in research on (a) and (b) should be undertaken now.

In conclusion I stress that studies on structure-activity are not by themselves sufficient to incriminate a chemical beyond a shadow of a doubt for a given chronic toxic effect; it needs strong support from what I have called short-term assays, i.e., (b above, and where at all feasible from (c) animal bioassays and (d) epidemiology. A vast amount of animal data is in the literature, but much of this has been incorrectly reviewed or not reviewed at all. Critical reviews of the various chemical groups are sorely needed.

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FORECAST OF POTENTIAL TECHNOLOGY CHANGES THAT MAY IMPACT TOXICOLOGICAL TESTING REQUIREMENTS

ICAIR Task Assignment No.: 107
Task Assignment Title: Impact of Changes

Task Assignment Report

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IMPACT OF CHANGES

The following questions/issues have been answered from the viewpoint of a biostatistician involved in the area of research and analysis on animal and human toxicological data. The area of biostatistics and data analysis has gone through considerable advances in methodology for analyzing toxicity data, particularly, animal chronic toxicity data within the last five years and this trend will continue in the next five to ten years. The impact of these advances are reflected in my answers to the following questions/issues. I have not attempted to answer all questions/issues suggested in those sent to me for examination, but only those which are directly related to biostatistics and data analysis which might arise from the anticipated answer. My numbering in this report is not the same as the questions sent.

Questions/Issues for Forecasting Agency Changes

1. What impact will the new administration have on regulations dealing with mammalian toxicology testing?

   The new administration, with an assumed greater emphasis on use of chemicals having possible toxic side effects when an economic benefit outweighs small health risks will, I believe, allow for controlled limited use of toxic chemicals, when chronic toxic tests show minimal health effects. The impact of this on mammalian toxicology testing will allow for tests which generate more data to fully assess toxic effects.

2. What may foreign countries do that would impact mammalian toxicology testing regulations?

   A move for unified guidelines for analysis of the carcinogenic effects in long-term animal experiments has recently been published by International Agency for Research on Cancer (1980). This document calls for more sophisticated analysis of such experiments. This will require more carefully designed mammalian toxicity testing including perhaps, longer term tests, serial sacrifice, measurement and analysis of graded and/or multiple tumors, and consideration of age of onset of tumor. Such analysis will call for more fully detailed experimental protocols.

3. Is there a trend toward: self-regulation, state regulation, federal regulation and/or civil/criminal court action?

   Based on my personal experience I see two definite trends. One is toward more specific and detailed federal guidelines which generate self-regulation by industry, etc., using guidelines as a basis. The second trend is toward more civil/criminal court action. As rules are promulgated and enforced, I see a definite motion toward court resolved solutions. As a biostatistician, the need for testimony on data analyses by other biostatisticians and myself has grown considerably in the last three years. I expect this to accelerate in the next five years.

4. Is there a trend toward self-regulation?

   There is a trend toward self-regulation in response to more specific federal guidelines. Management assesses the economics of any use of possibly toxic chemicals more carefully prior to any full scale development.

5. Is there a trend toward interagency (federal) cooperation and coordination on regulations dealing with toxicology testing?

   Yes, I see a definite trend toward interagency cooperation, coordination and guideline writing. As an illustration of this, consider the Report of the Interagency Regulatory Liaison Groups, Work Group on Risk Assessment (1979). Such interagency joint reports will lead, I believe, to more uniform rules, both for regulation and for the design and analysis of carcinogenicity testing.

6. Is there a trend toward more public involvement in the development of regulations that deal with toxicology testing?

   Yes. As evidence of this consider the recent debates over the Water Quality Criterion Documents by EPA in which there were public hearings, scientific review with industry and consumer interests represented, and a long process for reworking of criterion to reflect public interest. Also, both the saccharin and nitrite problems have resulted from vast public involvement encouraging review by special panels set up by the National Academy of Sciences at the requests of FDA, EPA, etc.

7. Are the laws (new and amended) providing more specific directions for regulations?

   Yes. This is particularly true of the Toxic Substances Control Act, the Clean Air Act and the Fungicide, Insecticide, Fumicide, and Rodenticide Act. These have and will continue to have a tremendous effect on toxicological testing. They call for balancing risks and benefits. As such, more detailed toxicological testing is required to do an appropriate risk assessment. That is, more data on toxicology must be gathered and analyzed.

8. Is the trend in the development of toxicological regulations toward more scientific involvement, legal involvement or consideration of economic aspects?

   I see a growth in all three areas. As the complex nature of carcinogenicity of many substances is becoming more detailed, this requires complex scientific involvement in any risk assessment. Because of the ubiquity of toxic substances, this also involves many economic and legal questions in risk/benefit assessments.

9. Are regulations becoming more universal?

   No. On the contrary, regulations will become more specific as scientific knowledge and data increase.

10. Will regulatory changes occur more rapidly?

    Changes will not occur more rapidly. In fact, there will be a backsliding, in that vague regulations will be dropped or ignored and only specific, well-understood scientifically defensible regulations will be

featured. Because of the complexity, such regulations will be added at a slower pace.

11. How do advancements in technology for conducting toxicological testing impact regulations?

    There have been great advances in the statistical analysis of toxicity data in the last five years, and the rate appears to be increasing. The net result of this is that time-to-response data is becoming more crucially involved as an important factor. This will require that toxicological testing protocols will call for serial sacrifice experiments over a larger range of doses. A recent report by the Scientific Committee of the Food Safety Council (1980) details a complex series of toxicological data to be gathered for such an assessment.

Questions/Issues for Forecasting Toxicological Testing Technology Advances

12. What is the impact of advances in statistical and mathematical approaches to toxicological testing requirements?

    The main impact of advances in statistical and mathematical approaches are twofold. Future experiments will be carried out at more dose levels and involve gathering time-to-tumor data by serial sacrifice. The reason for this is that current work in mathematical modeling and experimental design has shown that to get good information on dose-response and time-to-response and time-to-tumor, one must design a more complex chronic animal test than is typically carried out currently. Furthermore, such information is absolutely vital if one wants to do an analysis other than a simple-minded linear extrapolation of data. Such a simple procedure as linear extrapolation has shown itself to be quite insufficient for certain risk assessment questions. Thus, the statistical and mathematical models, particularly the multistage model of carcinogenises incorporating time-to-tumor require a protocol for gathering data at a variety of doses and at various serial sacrifice times. The extreme importance of time-to-tumor has been recognized as a result of the recent $ED_{.01}$ study carried out at the National Center for Toxicology Research.

13. What is the impact of computers and data acquisition equipment on toxicology testing?

    The current impact is that large data sets with complex statistical analysis using non-linear maximum likelihood techniques can now be done routinely and are relatively inexpensive. Also on-line monitoring of toxic side-effects in animal testing now being explored using monitoring computers will lead to much more useful and plentiful data in the near future.

14. What will the future value of toxicological data developed under present day standards be?

    The future value of current day data is limited. Most toxicological data gathered today can answer limited questions regarding toxic endpoints. For example, whether or not a chemical can cause tumors. But more useful risk estimation requires more animal data at varying doses and points in time. Time is an important factor since late tumors are not as dangerous

and have less life-shortening risk than early tumors. Most experiments today lack such data and thus, are of limited value.

15. Will the centralized data base storage and retrieval mechanisms that are established and being developed permit any significant reduction in toxicological testing requirements in the future?

    I see very little value to current data bases until further basic mechanisms and unifying principles and models for the carcinogenic processes are understood. This will include well understood biochemistry, DNA repair models, metabolic pathways for general classes of chemicals, etc. Thus, current data bases are, at best, of limited use.

16. What will be the future impact/role of epidemiology studies in human health hazard assessments?

    Epidemiology will always be useful as confirmation of *in vivo* toxicity in man, but its role in early warning of toxicity will be decreased by the short-term mutagenic test development and chronic animal testing. Data analysis of animal and short-time tests will be more relevant than epidemiology in the coming years. I do not think confirmatory human data will always be required to supplement results from animal tests.

17. What will be the projected speed for acceptance of technology changes for toxicity testing in your specific discipline area?

    Acceptance of mathematical models and statistical risk estimates based on them is still quite new in the field of toxicity testing. But the realization that simple ideas of linear extrapolation, safety factors, etc., are no longer adequate is causing the use of more complex mathematical models. A recent paper by myself entitled, "Quantitative Risk Assessment," Van Ryzin (1980), discusses this issue in regard to four models. I have had a tremendous response to this article and I sense a growing interest in the use of mathematical models. There is a reluctance by less quantitatively oriented biological and regulatory people to use such models, but the necessity for more realistic risk assessment in the future will erode such resistance.

18. Forecast the level of support for toxicology research and development, especially in the areas of basic research and its impact on technology advancement.

    Support in the area of basic research in mathematical and statistical methods for analyzing toxicity will easily double within the next five years. Its impact will be a necessity for more complex protocols to gather the necessary data in toxicity tests.

19. What federal agency/organization programs are likely to be pacesetters for development in toxicological testing technology?

    I foresee the National Institute of Environmental Health Sciences, the National Toxicology Program and private organizations such as the Society of Toxicology and the Food Safety Council as some of the main forces behind developing more useful toxicological testing technology; especially

in the areas of mathematical modeling and statistical methods of risk assessment. The EPA and NIOSH have, I felt, sought simple, easy-to-regulate solutions which, unfortunately, oversimplify the necessary science and testing methodology which should be encouraged.

20. Forecast the impact of mutagenic screening and other screening techniques on overall toxicology testing requirements.

    I do not see mutagenic tests as a basis for quantitative risk assessment for many years. However, they will be used for early detection screening and prioritization schemes for animal chronic tests.

21. Will concern for synergistic effects due to exposure to multiple chemicals significantly impact short and/or long-term toxicological testing requirements?

    The impact of synergistic effects will be to require larger studies and more stringent risk assessments when synergisms are suspected. However, I do not see a full incorporation of synergistic effects into mathematical modeling for some time (not in the next five years), since even the understanding of, data analysis for, and risk assessment of a single carcinogen alone is still a costly and difficult problem to do by toxicological testing.

22. Will there be a trend toward the consolidation of toxicology testing protocols?

    Yes, there will be some limited trend to study chemicals which behave biologically the same. However, the consolidation will be small in the near future.

23. What progress related to cancer research would impact toxicological testing requirements?

    I think basic research in the mechanisms of cancer induction will have tremendous effect. For instance, establishing if the carcinogen acts by direct binding to DNA, through one of its metabolites, through promotion of an existing process, etc., all will have a tremendous effect on test requirements to assess data and design protocols to answer these questions.

24. Forecast the impact and pace of developments in improved risk assessment techniques.

    Risk assessment techniques will see an explosion within the next five years. The question of carcinogenicity (yes or no) is not enough. When the carcinogenicity occurs (old age or not), what subpopulations are affected, how much in expected loss of life is associated with decreased/or increase use, and other such questions will be common parts of future risk assessments. The impact of this will be more carefully designed toxicity tests.

25. Forecast the availability of scarce personnel on the ability to perform toxicity testing.

I feel there will be a shortage of biostatisticians at the Ph.D. level who are qualified to design and analyze toxicity data. The numbers of such statisticians involved are few and are in great demand.

26. Evaluate the role of scientific/professional societies in promoting technology changes that will impact testing requirements.

The Society of Toxicology and Biometric Society are the two scientific groups having the most impact currently on changing testing requirements from a data gathering and statistical design point of view. Their role will continue in the future and their impact will be to call for better designed experiments over wider dosage and time schedules.

27. What will be the influence of current basic research investments on future technology?

The current basic research in biostatistics in the area of toxicity testing is development of models which incorporate time-to-tumor and dosage information in the model. Also, models such as the proportional hazard model, the Armitage-Doll multistage model and the multihit models all have received much research in the last three years. These models all require gathering data on multiple responses, covariate information, time-to-tumor, and extensive dosage data. This calls for design of more complex protocols and gathering more data.

28. What are the best analogies to toxicity testing technology?

The best analogies from the statistical point of view is that of the methodology used in life-testing in engineering and aerospace fields. An animal on a lifetime or chronic test is most like studying a machine part for failure (occurrence of toxicity is like a failure). The big difference is that controlling biological variables in designing the animal study is much less well understood. Much of the literature on life-testing is usable for toxicity statistical analysis when time of occurrence of the toxicity is relevant.

29. Will there be increased or decreased emphasis placed on toxicological effects which are reversible or irreversible?

The emphasis will be increased to determine the exact nature of the toxicity induced. Even irreversible toxicities need be clearly studied as to when they occur in the animal. An irreversible toxicity occurring late in life is quite different than one occurring earlier in life. Thus, there will be increased emphasis on determining the exact nature of the toxicological effect.

30. What technology changes are anticipated in the area of duration of toxicology testing studies?

The technology changes in the subchronic tests will be in the area of mutagenic and metabolic tests prior to a full scale chronic test. The Scientific Committee of the Food Safety Council in its report cited above (2) gave a detailed review of a system of tests that would be ideal. It

emphasizes more metabolic and genetic testing at the subchronic level. Furthermore, it calls for more extensive tests for chronic tests with many dose levels (5 or more) involved and, when possible, serial sacrifice of animal for time data.

31. Forecast potential advances in data evaluation and interpretation techniques that may permit improved communication of the results from toxicity testing to both decision makers and the general public.

    The main advantage is that new technology will allow more precise estimates of risk; that is, for which risk groups, for which age groups, and the meaning of risk versus benefit. The public is confused by "the everything causes cancer" syndrome and feels science has been confusing. As statistical methods and better chronic testing protocols are developed, more definitive notions of risks and their estimates will be forthcoming. This will help the public and decision makers make better and more informed risk/benefit decision.

32. What mathematical models among those currently in use will gain more acceptability?

    The one-hit model and the Mantel-Bryan procedure for risk assessment of chronic toxicity testing are much too simplistic and will become less used in the future. The multistage, Weibull, multihit, and proportional hazard model and their time-to-tumor generalization will all be used in the future much more frequently. See the Scientific Committee of the Food Safety Council Report, the paper "Quantitative Risk Assessment" by myself and the IARC report cited above for references and indication of the more sophisticated methodology to be employed in the future.

33. What notions will be introduced into risk assessment by mathematical models that will have a profound effect on toxicity testing?

    This question was posed since I have noticed a definite swing away from the single notion of estimating the virtual safe dose; i.e., that dose leading to a lifetime risk of, say 10^{-6} or 10^{-8}. Ideas of late dose risk, median lifetime risk and residual lifetime risk, all which involve both time of occurrence of irreversible toxicity as function of dose as well as simple frequency of occurrence of the toxicity will become more meaningful in future risk/benefit assessments. The impact on toxicity testing will be more expensive and more complex tests. Due to limited testing capability nationwide, this will require more careful screening of compounds in subchronic and short-term tests.

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FORECAST OF POTENTIAL TECHNOLOGY CHANGES THAT MAY IMPACT TOXICOLOGICAL TESTING REQUIREMENTS

ICAIR Task Assignment No.: 107
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IMPACT OF CHANGES

1. Forecast equipment needs for laboratory toxicological testing.

   A need for automation; miniaturization; more durable nontoxic materials for bioassay equipment fabrication; upgrading of electronic components and circuitry (durability); personal monitoring devices applicable to human and animal models; remote sensors (internal and external body implants) and biotelemetry devices; artificial body organs/systems viz bioengineering; significant increases in analytical/detection capabilities of instrumentation; single instrument - multiple detection capabilities.

2. Forecast changes in analytical chemistry required for support of toxicological testing.

   Continued reduction in wet chemistry analytical procedures. Refinement of existing technologies, e.g., high-pressure liquid chromatography; gas chromatography; gas liquid chromatography mass spectrometry; electron impact and chemical ionization mass spectrometry; nuclear magnetic resonance; infrared and ultraviolet spectra analyses. Continued need for measurement of body burden metabolites, dose levels, stability, environmental exposures, threshold levels, and regulatory impact of standards, TLVs, etc., will mandate improved and new analytical procedures. The era of biological monitoring should trigger a wave of methods development (1).

3. What is the impact of advances in statistical and mathematical approaches to toxicological testing requirements (experimental design and data evaluation)?

   One would anticipate more scientifically sound and accepted data and potential for reduction in resources required, e.g., numbers of animals, species, duration of studies, in vitro vs. in vivo studies, acute vs. chronic assays, manpower. Examples of mathematical modeling are appearing with increasing frequency (2, 3, 4, 5).

   The impact of "modeling" and mathematical approaches is to many "in vivo" scientists equivocal in light of data such as presented in the report by Ramsey et al., cited above.

   (Technical information contained in the NIOSH "Handbook of Statistical Tests for Evaluating Employee Exposure to Air Contaminants," (6) may also be of value for inclusion in the task for "Forecasting Technology Changes.")

4. What is the impact of computers and data acquisition equipment on toxicology testing?

5. Will the centralized data base storage and retrieval mechanisms that are established or being developed permit any significant reduction in toxicological testing requirements in the future?

   Resources available for future research (manpower, facilities, dollars), and mass of data will mandate signficant development and utilization of computers, software, storage and retrieval systems, etc., for toxicity

testing and possible simulation of animal models through mathematical modeling. In reference 3, a section on "Biological and Biomathematical Methods in Efficient Animal Experimentation," and in reference 4, sections on data retrieval, data systems support, and automated and computer-assisted pathology support relate to this question. The NTP Annual Plan for fiscal years 1979 and 1980 specifically identify "Data Management and Analysis."

With the exception of mathematical modeling in lieu of mammalian bioassays, significant reduction in toxicological testing requirements is not anticipated. The classical routes of exposure/dosing will continue to be employed. Computerization should, in fact, increase the capacity and degree of testing through utilization of historical data bases.

6. Under the assumption that toxicological testing requirements will become more stringent (greater quality assurance), what will be the future value of toxicological data developed under present day standards?

   Quality assurance programs of the future will undoubtedly be refined and become more stringent than what is now identified under GLP regulations. In many instances data generated under present day standards will be tested and compared under the protocols/regulations of the future. With few exceptions, studies accomplished with today's technology and instrumentation by competent and conscientious scientists will stand the test of time and application.

7. What will be the future impact/role of epidemiology studies in human health hazard assessments? (Will confirmatory human data always be required to supplement results from animal tests?)

   Epidemiological studies are construed as applicable to animal and plant populations in addition to the general association with human cohorts.

   Confirmatory human data will not always be required to supplement results from animal tests. Epidemiological studies will be applied with a significantly greater frequency and in applications not now identified or recognized for use in animal bioassay programs. This is particularly true for occupational carcinogenesis, cancer risk assessment studies, teratogenesis and mutagenesis, and the development or denial of thresholds of biological response, cancer or otherwise (1, 3, 7, 8).

8. What will be the projected speed for acceptance of technology changes for toxicity testing in your specific discipline area?

   Acceptance of technology changes in toxicity testing, including the inhalation route of exposure will be rapid once such technology has been "field tested" by competent personnel/organizations and where applicable, meets guidelines or specifics of regulations and standards. At present a significant effort is being applied to develop new techniques for the standard Draize tests for cutaneous and eye irritation. Because of the heavy investment in hardware for inhalation toxicology studies, acceptance and substitution of new chamber designs, aerosol generation systems, etc., will be incorporated at a slower rate, e.g., evaluation of recently designed multi-tiered exposure is currently in progress by several investi-

gators. Computerization and automation of inhalation toxicology facilities will probably be the most significant new technology accepted and applied in this specific discipline.

9. Forecast the level of support for toxicology research and development, especially in the areas of basic research and its impact on technology advancement.

   Financial support for toxicology research and development, especially in basic research, will not be blessed with increased funding. Governmental funding may well decline during the next four and possibly eight years. Inflation will probably absorb any additional funds supplied by nongovernmental sources. New and increasing activity (and facilities) within industry will provide a new arena for toxicity testing and methods development, the CIIT being a prime example of such a program. The magnitude and rapidity of promulgation and enforcement of standards/regulations will definitely impact on levels of support. If promulgation is significant, which I doubt over the next 3-5 year period, support will be reflected in areas of applied vs. basic research and in areas of technological advancement (automation, computerization, integrated laboratory information systems, advances in <u>in vitro</u> testing system).

10. What federal agency/organization programs are likely to be the pace setters for developing advances in toxicological testing technology, i.e., National Toxicology Program, EPA, National Academy of Sciences, etc.?

    The National Toxicology Program will be a forerunner. This includes the NCI bioassay program relocated at the NIEHS, the NCTR studies on innovations in cancer risk assessment, and NIOSH and NIEHS inhalation toxicology and other bioassay programs. EPA is not the obvious federal agency leader in basic and applied research on evaluating chemicals in the environment. The CIIT will be a non-federal leader in toxicity testing and methods development. Programs will include mathematical modeling, use of new animal models, expanded use of <u>in vitro</u> systems, increased application of biochemical toxicology.

11. Forecast the impact of mutagenic screening and other screening techniques on overall toxicology testing requirements.

    The present application of such screening techniques, the intensity of interests to refine existing and develop new methods, research programs and funds dedicated to studies with screening techniques as a basis for generation of the toxicological data, and publication explicit on screening methodologies and their applications to toxicology, carcinogenesis, etc., demonstrate a valid and concerted scientific effort to apply such methods to the arsenal of testing requirements. Some will be validated and applied (1, 3, 7, 8, 11-15).

12. What is the anticipated pace at which these screening techniques are likely to receive full acceptance as the basis for regulatory actions?

    Full acceptance of the screening techniques as the <u>basis</u> for regulatory actions will be at a slow and tedious pace even though certain data derived from such procedures and methodologies have entered into the

decision making process and documentation for some OSHA and EPA regulations and standards.

13. Will concern for synergistic effects due to exposures to multiple chemicals significantly impact short- and/or long-term toxicological testing requirements?

    Synergistic and antagonistic effects, inhibitors, promoters and cocarcinogens are and will continue to impact on toxicological testing requirements. Man's environment will continue to be complicated by his own complex chemical contaminations, the effects of which, especially in terms of carcinogenesis and mutagenesis, will require life-time or extended duration of experimental animal exposures/post-exposure observation periods. Until such time as mathematical modeling, epidemiological and/or in vitro testing is equivocal and supplant or predict long-term impact and biological response to one-hit or a chronic chemical insult, present day testing requirements will persist.

14. Will there be a trend toward the consolidation of toxicology testing protocols?

    Consolidation of toxicology testing protocols will be minimal. Overlap of existing methods will be reduced as instrumentation and state-of-the-art for such are refined and thresholds of response are better delineated. Again, refinement and acceptance of in vitro testing procedures and application of biochemical toxicology may negate or modify the need for some of today's protocols but interest in specific types of biological response, target organ, metabolic route, sensory responses, etc., will require a battery of definitive testing procedures.

15. What is/would be the impact of focusing on the toxicological properties of chemical groups as opposed to specific chemical compounds?

    Focus will intensify on chemical groups vs. specific chemical compounds. "By analogy" will also receive more attention. Inducers of such considerations will be new and refined chemical/biochemical knowledge, particularly in metabolic pathways, pharmacokinetics, homeostasis of mammalian systems, genetics, and cellular membrane structure and function including cell molecular targets or species that are concerned with the execution of sustaining body functions (1, 3, 8, 12, 15, 16, 17).

16. What progress related to cancer research would impact toxicological testing requirements, i.e., in areas of defining specific causes for cancers, or in the treatment and "cure" of cancer?

    Progress in developing data on the toxicological/carcinogenic properties of chemical groups vs. specific compounds would reduce repetitive testing. Identification of cellular, subcellular, and molecular targets of foreign chemicals will shift emphasis to in vitro testing procedures. Identification of specific causes for cancers will initiate greater application of epidemiological studies, particularly on chemicals associated with the workplace, including the military.

17. Forecast the impact and pace of developments in improved risk assessment techniques.

Risk assessment is presently associated with long-term chronic toxicity tests and retro-and prospective epidemiological studies. Developments that can shorten such procedures, resulting in significant savings of dollars and manpower would impact heavily on today's programs. The present pace is slow. Mathematical modeling and the NCTR ED_{01} study are examples of today's effort. The NTP is approaching the question through development of new and refined short-term test methods that include microbial mutagenesis assays, mammalian cell transformations, immunology and neurobehavioral test batteries. NIOSH recommends future research for assessing reproductive hazards in the workplace in areas that include epidemiology, in vivo prenatal and neonatal exposures, in vitro teratogenesis test systems utilizing organ and whole embryo culture, rather than single cells, and metabolic activiation procedures (4, 7, 8, 10).

18. Forecast the role of structure activity relationships as they may replace certain toxicity testing requirements.

Improved techniques in analytical and clinical chemistry will have a significant impact on toxicological testing requirements. Resources for purchase of new equipment will be considerable. Improved sensitivity of analytical instruments will result in retesting or validation of historical data, or the generation of new, as applied to documentation and development of standards for human exposure to chemicals. Approaches and opportuni'y to expand toxicity testing protocols, particularly in pharmacokinetics and biochemical toxicology will be enhanced. The furtherance of toxicity data related to target organ concepts, thresholds/"no-effect level" for chemical exposures, biological monitoring methods, and generation of identity and purity profiles for test chemicals are all inherent in advances in analytical methods and instrumentation (18-21).

20. Evaluate the availability of laboratory animals on toxicology testing technology and/or requirements (controversies associated with use of dogs, scarcity and expense for use of primates, etc.).

The classical species of animals (rat, mouse, rabbit, guinea pig, hamster, dog, cat, monkey) will continue to be used for toxicology testing. New strains, inbred and outbread, will be developed. The subhuman primate, because of cost and availability will be used more sparingly and for specialized protocols. Recycling of this species for specific tasks will be considered more frequently. Domestic monkey bredding colonies should provide an increasing number of animals during the 1980's. Dogs will be considered more often as substitutes for monkeys in inhalation toxicology programs. New animal models will be tested for protocols now using the standard experimental species. Aquatic toxicology will receive a new impetus. Standard operating procedures and laboratory animal quality control programs will become more evident and mandated under GLP type regulations and guidelines associated with programs such as the NTP (1, 3, 8, 11, 22, 23).

21. Are advances/standardization of neurotoxicity and behavioral effects testing believed to take place in the relatively near future so that these

types of effects will have greater acceptance as a basis for establishing rules and regulations?

Advances/standardization of neurotoxicity and behavioral effects testing now have a low to medium level of acceptance for establishing rules and regulations. During the 1980's, acceptance should accelerate markedly. Technology and refinements in instrumentation and protocols to ensure reproducibility of data will contribute much to its acceptance. Early studies in the U.S.S.R. and equivocal scientific data did much to temper the acceptance of neurobehavioral testing in the Western world. Laboratories within the NTP are actively engaged in the development of new methods and in the routine use of existing methods for testing the behavioral and neurological effects of a variety of toxic agents. NIOSH, as a component of the NTP, and on its own initiative, is also actively engaged in such studies, in animals and with human volunteers (3, 7, 8, 11, 15, 17, 24).

22. Forecast the availability of scarce personnel (e.g., veterinary pathologists) on the ability to perform toxicity testing.

Today's climate for increased toxicological testing, regulations and guidelines will continue. Creation of an imbalance in supply and demand for a number of disciplines inherent to the conduct and interpretation of such testing was inevitable. The shortages of qualified personnel, e.g., veterinary pathologists, inhalation toxicologists, neurotoxicologists, pharmacokinetists, will advance well into the 1980's or beyond. Shortcomings in personnel needs for toxicology programs were identified as early as 1960. Specialization within the scientific discipline of toxicology has created an even wider divergence of skills and shortages than imagined or projected in 1960. Manpower shortage in both professional and technician levels of skill was identified in 1977 as the most significant obstacle to expansion of programs in toxicity evaluation by the inhalation route of exposure. The Society of Toxicology has long advocated and supported professional and academic training programs. This must be accelerated in order to satisfy the present and future needs of government and industry testing programs.

23. Evaluate the role of scientific/professional societies in promoting technology changes that will impact testing requirements.

Members of such societies are more often than not the leaders in disciplines that influence or dictate testing requirements. Thus, the role of such organizations will impact heavily on changes and advances in technology/state-of-the-art, e.g., The Society of Toxicology, American College of Vet. Pathologists, American College of Toxicology, New York Academy of Sciences, American Industrial Hygiene Association, Society for Occupational and Environmental Health, but to mention a few and not forgetting the National Academy of Sciences.

Promotion will be through education programs, symposia, incorporation of new technology in research protocols, presentations at national and international scientific meeting and through the public news media.

24. What will be the influence of current basic research investments on future testing technology?

Current basic research investments on future testing technology will be reflected by resources available or anticipated in the next 2-5 years. Personnel ceilings, facilities, equipment investments, government and non-government funding will all influence progress and investments in the 1980's. The NTP and programs of the EPA and military, the CIIT and other non-government entities are heavily endowed to new basic research programs. Financial support is needed to trigger the avalanche of new ideas and technology and their applications to research. The economic climate of January, 1980 and the near future is cloudy.

26. Forecast the degree of concern for the safety and health of persons performing toxicity testing and what impact that will have on future testing resource requirements.

In my judgment, the degree of concern for the safety and health of persons performing toxicity testing is 10 on a scale of 1 to 10. Disregard for such concern is to a large measure responsible for many of the overdue health and safety regulations now promulgated or recommended. The impact on future testing resource requirements will be heavy in such areas as providing personal safety equipment and apparel, containment of test chemicals at site of testing, minimizing or eliminating effluents into the outside environments (air, water, sewers), hazardous waste disposal including chemical-contaminated animal carcasses, and bedding. Regulations under OSHA, EPA, RCRA, TSCA, FIFRA will require personnel for administration and compliance.

27. What impact will medical treatment/advances have on reducing the concern with certain adverse toxic effects?

It is my opinion that medical treatment/advances deserve minimal or no consideration as to impact on reducing the concern with certain adverse toxic effects. Therapeutic measures are not answers to prevention. Prophylactic measures are at best stop-gap and often of short duration. "An ounce of prevention is worth a pound of cure."

28. Will there be increased or decreased emphasis placed on toxicological effects which are reversible or irreversible?

Emphasis on toxicological effects which are reversible or irreversible will be identified wth specific areas, e.g., biochemical toxicology, pharmacokinetics, biodegradability studies, immune assessments, tolerance mechanisms. Recognition/identification of such effects will be increased and emphasis shifted upward as new toxicology testing technology is generated, applied and validated in future research programs. Studies of occupational diseases/industrial toxicology and short-term, acute exposure identified with military chemicals and operations should also receive enhanced attention to reversible/irreversibility.

29. What technology changes are anticipated in the areas of routes of exposure: (a) inhalation, (b) oral, (c) dermal, (d) ocular, (e) other?

Present day routes of exposure will continue in force as will the technologies presently employed. More emphasis is anticipated on evaluation of intralaboratory and interlaboratory reproducibility of defined protocols. GLP's and quality assurance programs will contribute to this factor. Lifetime animal inhalation studies will be more evident in defining oncogenic and mutagenic responses. The use of aged animals at the initiation of a study will receive more attention, e.g., start with 12-month old rats. New animal models will be introduced as will prescreening procedures to aid in establishing priorities for in-depth studies. Dermal and ocular testing procedures may be modified (see Question 8, re Draize test). Routes and methods of testing for aquatic toxicology and behavioral/neurotoxicity will be refined. Testing standards will be better defined and required for acceptance of generated toxicity data.

30. What technology changes are anticipated in the area of animals used for toxicology testing, (a) rodents, (b) primates, (c) other animals?

Attempts to develop and define new animal models will continue as always. Aquatic and avian forms will receive more attention. Smaller forms of subhuman primates, e.g., marmosets, lemurs, have seen limited use in the past and more applications may be attempted in light of the cost and availability of Rhesus and Cynomolgus monkeys. Rodents will continue to dominate as the species of greatest use. Pharmacokinetic/metabolic pathways studies and reproductive toxicology may promote the greatest interest in development of new animal models. Laboratory animal production and quality control will receive significant attention (1, 8, 22, 29).

31. What advances are likely in non-animal testing that will reduce the amount/extent of animal testing?

Continued evaluation and validation of *in vitro* test systems will reduce the extent of whole-body animal testing. Greatest impact will be in mutagenic, teratogenic, and carcinogenic assessments. Standardization of tests, e.g., Salmonella/microsome plate assay and tissue culture techniques have already impacted on *in vitro* vs. *in vivo* testing. New and improved mathematical modeling and biometric design of experiments will also reduce the scope of animal testing (1, 7, 8, 10, 11, 13, 14).

32. What technology changes are anticipated in the area of duration of toxicology testing studies, (a) for acute effects, (b) for sub-chronic/subacute eff cts, (c) for chronic effects?

Development of more specific screening methods for carcnogenic, mutagenic, and teratogenic chemical risks will shorten duration of studies, e.g., sub-cellular (purified enzymes, DNA, RNA) test systems; isolated cells (blood components, bacteria, cell/organ cultures); tissue sections; isolated whole organs; intact embryonic systems (fertile eggs). Lifetime (chronic) bioassays will use designs that vary the age of animals exposed and the duration of exposure. Greater use of epidemiological data should provide risk assessment data that will influence type and duration of studies (1, 8, 10, 11).

33. Will it be required to duplicate the route of actual human exposure during future toxicity studies with animals?

The route of administration or exposure generally should be the same as the route by which human exposure occurs. Consideration should be given to the comparability of absorption, retention, distibution, metabolism and excretion of the chemical in the test animal species and in man in order to maximize extrapolation of results and data in terms of significance to man. Some routes may be utilized because they have special advantages directed toward merely screening for certain biological activity (carcinogenicity, mutagenicity, hypersensitization) with no intent to facilitate quantitating the hazard to humans (1, 10, 11).

34. Will it be possible to use non-inhalation toxicology data to predict human health hazards associated with inhalation exposures? Is this due primarily to economic constraints or is it likely to be a technically "acceptable" alternative?

While not a technically acceptable alternative, non-inhalation toxicology data can be used in some instances to assist in predicting health hazards associated with inhalation exposures. There are many compounds to which man is exposed by several different portals of entry to the body. It is essential in using the non-inhalation data that consideration is given to specific properties of the chemical, e.g., solubility, absorption, target organs, metabolism. Future emphasis should be given to developing comparative data, particularly in metabolic pathways, and to developing other sensitive biochemical indices of pulmonary toxicity, as well as improved new conventional measures of respiratory function (11).

35. Forecast potential advances in data evaluation and interpretation techniques that may permit improved communication of the results from toxicity testing to both decision makers and the general public (i.e., will the creditability of the scientific community to predict human health hazards improve, deteriorste or remain at its present level).

Creditability of the scientific community to predict human health hazards will improve dramatically. Innovations in cancer risk assessment such as the NCTR ED_{01} study is but a first step in such scientific attempts. Epidemiology, new and refined <u>in vitro</u> testing procedures, pharmacokinetics, neurotoxicology, analytical chemistry and environmental monitoring methods will require new generations of data management systems for data acquisition, storage, and retrieval; data reduction and analysis; management tracking and control, and appropriate statistical methodologies (1, 4, 8, 10, 20, 30, 31).

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TR-477-14-12

FORECAST OF POTENTIAL TECHNOLOGY CHANGES THAT MAY IMPACT TOXICOLOGICAL TESTING REQUIREMENTS

ICAIR Task Assignment No.: 107
Task Assignment Title: Impact of Changes

Task Assignment Report

by

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IMPACT OF CHANGES

Assumptions

1. It is assumed that there will be no change made by Congress in the Food, Drug and Cosmetic Act in terms of the basic concept requiring the establishment of safety of food, drugs and cosmetics before they may be regulated. Changes which define conditions under which materials may be used would not invalidate these assumptions. As an example, a change in the absolute nature of the Delaney Clause would not affect the toxicological requirements for safety testing.

Technology Changes to Satisfy FDA Requirements

2. What will be the changes in requirements for analytical chemistry to toxicological testing?

   The Good Laboratory Practice regulations (43FR59986, December 22, 1978) require chemical analysis of test mixtures used in feeding or other experiments to insure that the intended levels of exposure have been attained. This is a minimal requirement. Good scientific practice indicates the need for more complete chemical characterization of materials to which test animals are exposed in order to insure proper interpretation of effects noted.

3. What will be the future value of toxicological data developed under present day standards when considered in the light of stringent future requirements?

   All data have some value. The real question, in my opinion, is how much regulatory weight could be given to the data from a particular experiment. This is a matter of judgment (or better yet, guidelines) and would depend upon how nearly the study protocol approached the instant requirements and to what extent the data could be validated.

4. What will be the projected speed for acceptance of technology changes for toxicity testing?

   If one looks at the requirements for toxicological testing as published in 1959 by the FDA [1] in comparison to more recent FDA expressions in this regard [2,3,4] it becomes apparent that over the period of some 20 years, basic, regulatory toxicology requirements have undergone little real change. From my own experience the major emphasis has been to increase the data base without changing this type of experimental work. The FDA, together with other Federal agencies (the Interagency Regulatory Liaison Group), is now in process of codifying their requirements. The regulatory process by its nature is slow. If the progression outlined here has any validity as an index to the future - and in my opinion it does - I believe that technological advances will be introduced very slowly and can be planned for adequately.

5. What is the anticipated level of support for toxicological research and development, especially in the areas of basic research and its impact on technology advancement?

The Bureau of Foods published a notice of availability of its Draft Research Plan (45FR18365, 18480, March 21, 1980) for public comment. This is an extremely broad plan including a number of areas which, if passed, will affect technology. I do not believe, however, that it will affect toxicological testing to any significant degree within the next five to ten years.

6. What will be the impact of mutagenic screening and other screening techniques on overall toxicology testing requirements?

   I assume first that these techniques are screens for carcinogenic potential. From a regulatory point of view the effects, in my opinion, will be minor. This is based on the fact that they serve only to indicate, not delineate. Thus, a no-effect finding in screening tests will not provide the needed assurance of non-carcinogenicity and will not do away with the usual animal test assays. In any event, should mutagenic or other techniques become absolute, i.e., be accepted as definitive evidence as to carcinogenic potential from a regulatory point of view, it would at most do away with the present requirement for the life-time rodent studies.

7. Will concern for synergistic effects due to exposure to multiple chemicals significantly impact short- and/or long-term toxicological testing requirements?

   From the FDA point of view, especially as it pertains to foods, I do not believe so. The manifold problems, the costs and the time which attend the testing today of even a single chemical make it unlikely that the Agency will be able to demand testing in this area within the foreseeable future.

8. What is/would be the impact of focusing on the toxicological properties of chemical groups as opposed to specific chemical compounds?

   Although this would be worthwhile in a general sense, I do not believe that regulatory decisions would be made on this basis for individual compounds in the foreseeable future. While toxicological requirements have been modified for members of some classes of substances - modified starches for example - this is the exception rather than the rule.

Potential FDA Regulatory Examples That May Impact Toxicology Testing Requirements

9. What may foreign countries do that would impact mammalian toxicology testing regulations?

   The FDA is working with the Organization of Economic Cooperation and Development (OECD) to codify the OECD toxicology requirements. It is likely that the OECD and FDA requirements will be substantially similar.

10. Is there a trend toward more interagency (federal) cooperation and coordination of regulations dealing with toxicology testing?

It is clear that a beginning in this direction has been formalized by IRLG. In my view this has become a necessity in order to minimize duplication of toxicology requirements. What each Agency does with the data will depend on the law(s) under which it operates.

11. Is there a trend toward more public involvement in the development of regulations that deal with toxicological testing?

The FDA in the normal course of promulgating regulations publishes proposals and proposed regulations for comment. All elements of the population may respond. Based upon what I have seen at the FDA, it is quite clear that over the past fifteen years there has been a marked increase in the participation of the consuming public in the regulatory process both in terms of public interest groups as well as individuals.

12. Are the laws (new and amended) providing more specific directions for regulations, specifically, those regulations dealing with toxicological testing?

My direct experience with amendments to Food, Drug, and Cosmetic laws has related to proposed amendments to the cosmetic provisions of the FDA Act. There have been included specifics for toxicology testing, but these specific requirements would not introduce new technology, and none of these proposed amendments were enacted. In my view, Congress is unlikely, over the long range, to enact legislation on a specific basis, i.e., on a test-by-test basis.

13. Is the trend in the development of toxicological regulations toward more scientific involvement, legal involvement or consideration of economic aspects?

As I understand this question I believe that the regulations and/or regulatory actions have been occurring more recently because of the pressures of legal involvement or public pressures. FDA's cyclic review is a case in point. It will be undertaken at this time, I believe, largely in response to Congressional and public pressure. It occasioned the need to establish a set of guidelines for determining testing priorities; it required formally establishing guidelines for protocols. To some extent it may have had some impact on the participation in the IRLG. In sum, I believe that legal considerations provoked by public pressure have a good deal to do with today's regulatory activity.

14. Are regulations becoming more universal (i.e., no exceptions), or are they becoming more special-case oriented?

With respect to FDA, Congress has reacted during the past four years with special case legislation. Specifically this occurred in the case of saccharin. There was congressional reaction more recently with respect to nitrites. This occurred because these compounds were perceived as uniquely important. I do not believe this represents a trend. Rather, the ultimate legislative action will be to modify in a general way those aspects of the law perceived as offensive.

15. Will regulatory changes occur more rapidly or at about the same pace as in recent years?

It is unlikely that there will be a number of changes relating to toxicity testing due to formalization of requirements. These will be, I think, in the form of detailed guides. They are likely to appear to be of relatively rapid occurrences. If so, it will be because of a large expenditure of special effort. The regulatory process, being what it is, usually limits the speed at which regulatory action can occur.

16. How does the natural economic picture impact regulations dealing with toxicity testing requirements?

Regulatory agencies are not likely to alter requirements for toxicity testing based on the economic picture. That would be bad science. Safety testing is carried out using what a consensus of scientific opinion considers to be good testing methodology. To do otherwise would be equivalent to burying one's head in the sand.

17. How do advancements in technology for conducting toxicological testing impact regulations?

Technological advancements will ultimately impact on regulatory requirements. It should be noted, however, that the pace at which they impact will be necessarily slow. Actions taken by a regulatory agency can always be contested legally. Because of this, any action taken by an agency must ultimately be supportable in a court of law. This then requires that the scientific basis for that action be well supported by a preponderance of reputable scientific opinion.

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REVIEW OF FORECASTS OF POTENTIAL TECHNOLOGY CHANGES THAT MAY IMPACT TOXICOLOGICAL TESTING REQUIREMENTS: NEUROTOXICITY AND BEHAVIORAL TOXICITY

ICAIR Task Assignment No.: 107
Task Assignment Title: Impact of Changes

Task Assignment Report

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IMPACT OF CHANGES

Introduction

The emphasis of this report will be on neurotoxicity and behavioral toxicity. The framework will be the set of Candidate Questions/Issues for Forecasting Toxicological Testing Technology Advances, included as Appendix 1 to this report. Some of these have been lumped together for emphasis. Political considerations are not taken into account.

Answers to Questions/Issues for Neurotoxicity and Behavioral Toxicity

Question 25

The term "behavioral toxicity" was coined during the mid-1950's[1] to describe the parameters that needed to be assessed for the prudent and effective use of psychotropic agents. This continues to be germane and is extended to chemical substances to which subjects are both voluntarily and non-voluntarily exposed for non-therapeutic purposes. Therapeutic purposes are defined as therapeutic regimens recommended and monitored by professional personnel. The other fundamental framework is the attention to the six (6) basic "whats" of pharmacology: 1) what chemical(s), 2) what dose, 3) what subject(s), 4) what conditions, 5) what route of administration, and 6) what effect(s).

Question No. 21

Beginning with 6 (what effect) first, neurotoxicity may be defined as structural or baseline changes whereas behavioral toxicity defines the complex modulation of neural and other physiological systems. Since the nervous system does not replace neuronal loss, exposure to chemicals or conditions that cause such losses may be monitored by a variety of conventional techniques. These include morphological approaches (cell counts, staining characteristics, physical alterations) and physiological approaches (reflex studies, spontaneous electrical activity, evoked responses). Behavioral evaluations include anecdotal behavior (checklist defined repertoires with or without intraspecies interactions), species preservational behavior (sexual behavior, aggression, interspecies interaction) and conditional behavior. Advances will come with the generation of data bases that allow insights into mechanisms and allow interspecies comparisons. It will be important to generate normative baselines as well as to evaluate the aging process and genetic drift.

Question No. 29

The route of administration and duration of exposure to the substance(s) is an important parameter. Technology advancements are needed to provide flexible and precise systems for the deliverance of airborne substances under a wide variety of conditions. The technology is at hand to evaluate other routes of chemical delivery.

Question Nos. 30 and 33

The conditions under which the exposures by all routes take place should bear some relationship to human exposure possibilities and under surrogate conditions.

For example, the work force will probably shift toward automation-based manufacture with closed systems. Thus, changes in the precision of responses rather than qualitative changes would be the monitoring endpoints for setting acceptable limits and probabilities for reversibility of changes. When evaluating other interactive vectors of change, again realistic surrogates must be kept in mind -- fatigue levels, nutritional status, previous and intercurrent drug history.

Question No. 21

The subjects are of major importance in the definition of neural and behavioral toxicity. Unit costs have dictated species choice with little regard for planned interspecies comparison or appropriateness. When multidisciplinary approaches to a problem are employed, the usual route is reductionism to the most simple systems possible to cut across all disciplines. However, when behavior is involved, little has been done to develop monitors to allow the upward transferance of data to more complex systems, organisms or interactions. Further, genetically managed species preset conditions of drug metabolism, special kinetics and neural and behavioral repertoires. The conditions vary with the aging process and with genetic drift. Finally, anthropomorphic transfer of data is difficult under all circumstances, but generality of findings across species lends more credance to each finding, whether positive or negative.

Question Nos. 1, 2, and 19

The substance-bound parameters are where the major technical advances will come. Advances in insights and predictability will be defined by how the technologies are employed. It is essential to identify the chemical species causing the biological change. Radiolabelling coupled with GC/MS analysis should greatly advance this area. Such data would enable pharmacokinetic analysis, using the route of administration as a variable, to define the influence of the uptake route on the nature and amounts of the chemical species and the physiological handling.

Question No. 17

Risk assessment for neural and behavioral toxicity is not well accepted at this time, except for the induction of epileptogenic processes. Advances could be made by the judicious use of primate models coupled with efforts to translate laboratory concepts into appropriate models of human performance situations. As intimated above, today's concern is with accidental or non-voluntary exposure to substances. This neglects voluntary use or exposure to powerful substances that affect short and long term behavior and that would certainly interact with chemical substances to which the subject may be exposed on a non-voluntary basis. An interesting approach to risk assessments that allows for multicompartment models has been recently published by T. C. Campbell[2]. While that report deals with carcinogenic models it also allows for multicompartment analysis.

Question No. 7

Epidemiology thus far has been of value only on an ad hoc basis. The quality of epidemiological prediction or models depends on the quality of the input. The general estimate is that less than 10% of the deaths in the country per annum are subjected to post mortem examination. Those that are examined are not subjected to detailed analyses for specific causative factors. Detailed consequences may be evaluated for a specific exposure; however, the normative denominators continue to be missing. A similar problem appears in the DEAA Drug Awareness Warning Network in that heresay evidence is given weight equal to objective detailed analyses. Future development of more effective reporting of all aspects of public health problems is desperately needed.

Question Nos. 6 and 22

The problems of shortages of personnel are intimately tied to support levels for both basic and applied research -- let alone for monitoring purposes. Abelson points out in a recent editorial[3] the general shortage of scientists and engineers. Shortages will continue without research dollars to give continuity and long term stability to training programs. At another level, the real question is training for a specific aspect or task. R/D capabilities demand a flexible, broadly trained work force that deals with problem solving and basic research. Channeled training with stultifying credentialing implies a monitoring or empirical philosophy where the quality of detailed performance is presumed to be the product of licensure procedures. Research workers have always relied on the quality of their work coupled with sustained effort allowing multiple corroboration to serve as the testing foil. The overregulation of the workplace, of scientific accreditation and of scientific performance assures constancy but minimal progress with little likelihood of breakthroughs.

Question No. 23

Professional and scientific societies are increasingly concerned with training in their own and related disciplines. The accreditation or licensure impetus is felt to be regulatory-based rather than scientifically-based. The usual problem is that rules and procedures designed to govern the market place spill over into the research facilities and academic institutions. This will ultimately limit the originality by a preponderant effort and attention to brute-force standardization at all costs despite the obvious need to maintain flexibility. The GLP act and rules are a case in point. The recently instituted boards in toxicology are an extension in personnel matters. Yesterday's questions will be examined and well codified. Tomorrow's questions will await serendipity.

Question Nos. 3 and 4

[illegible]puters, data processing and new approaches to modelling through statistics [illegible]matics fall into a class wherein the non-linearities of biology and [illegible] should be forcing new efforts. The most optimal setting would be [illegible] coupled on a regional basis.

Question No. 27

The medical contribution to toxicology resides in the development of antidotes or reversal strategies, once a toxicology event has been fully characterized. This would need multiple levels of discourse, in the neural/behavioral area from chemical interaction to retraining, to contain and compensate for irreversible losses. If there is significant progress in defining normative populations and model systems in ten years, will there be a significant reduction in the need for toxicological testing or evaluations? I think not. Again, the biology is drifting generally modulated by the chemical environment. Environmental and social stresses will interact with the biological processes to force continued monitoring -- hopefully not of details -- but of processes. It is hoped that the focus would be on algorithms to characterize and to problem-solve for the unexpected result.

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1. NAS. 1959. National Academy of Sciences. Psychopharmacology: Problems of Evaluation. NAS/NRC Publ. 503.

2. Campbell TC. 1980. Fed. Proc. 39:2467.

3. Abelson PH. 1981. Science 211:4478.

APPENDIX 1

CANDIDATE QUESTIONS/ISSUES FOR FORECASTING TOXICOLOGICAL TESTING TECHNOLOGY ADVANCES

1. Forecast equipment needs for laboratory toxicological testing.

2. Forecast changes in analytical chemistry required for support of toxicological testing.

3. What is the impact of advances in statistical and mathematical approaches to toxicological testing requirements (experimental design and data evaluation)?

4. What is the impact of computers and data acquisition equipment on toxicology testing?

5. Under the assumption that toxicological testing requirements will become more stringent (greater quality assurance), what will be the future value of toxicological data developed under present day standards?

6. Will the centralized data base storage and retrieval mechanisms that are established or being developed permit any significant reduction in toxicological testing requirements in the future?

7. What will be the future impact/role of epidemiology studies in human health hazard assessments? (Will confirmatory human data always be required to supplement results from animal tests?)

8. What will be the projected speed for acceptance of technology changes for toxicity testing in your specific discipline area?

9. Forecast the level of support for toxicology research and development, especially in the areas of basic research and its impact on technology advancement.

10. What federal agency/organization programs are likely to be the pace setters for developing advances in toxicological testing technology, i.e. National Toxicology Program, EPA, National Academy of Sciences, etc.?

11. Forecast the impact of mutagenic screening and other screening techniques on overall toxicology testing requirements.

12. What is the anticipated pace at which these screening techniques are likely to receive full acceptance as the basis for regulatory actions?

13. Will concern for synergistic effects due to exposures to multiple chemicals significantly impact short- and/or long-term toxicological testing requirements?

14. Will there be a trend toward the consolidation of toxicology testing protocols?

15. What is/would be the impact of focusing on the toxicological properties of chemical groups as opposed to specific chemical compounds?

16. What progress related to cancer research would impact toxicological testing requirements, i.e. in areas of defining specific causes for cancers, or in the treatment and "cure" of cancer?

17. Forecast the impact and pace of developments in improved risk assessment techniques.

18. Forecast the role of structure activity relationships as they may replace certain toxicity testing requirements.

19. Forecast the role/impact of improved radiolabeling techniques and chemistry (analytical and clinical) on toxicological testing requirements.

20. Evaluate the availability of laboratory animals on toxicology testing technology and/or requirements (controversies associated with use of dogs, scarcity and expense for use of primates, etc.).

21. Are advances/standardization of neurotoxicity and behavioral effects testing believed to take place in the relatively near future so that these types of effects will have greater acceptance as a basis for establishing rules and regulations?

22. Forecast the availability of scarce personnel (e.g. veterinary pathologists) on the ability to perform toxicity testing.

23. Evaluate the role of scientific/professional societies in promoting technology changes that will impact testing requirements.

24. What will be the influence of current basic research investments on future testing technology?

25. What are the best analogies to toxicity testing technology?

26. Forecast the degree of concern for the safety and health of persons performing toxicity testing and what impact this will have on future testing resource requirements.

27. What impact will medical treatment/advances have on reducing the concern with certain adverse toxic effects?

28. Will there be increased or decreased emphasis placed on toxicological effects which are reversible or irreversible?

29. What technology changes are anticipated in the areas of routes of exposure: (a) inhalation, (b) oral, (c) dermal, (d) ocular, (e) other?

30. What technology changes are anticipated in the area of animals used for toxicology testing, (a) rodents, (b) primates, (c) other animals?

31. What advances are likely in non-animal testing that will reduce the amount/ extent of animal testing?

32. What technology changes are anticipated in the area of duration of toxicology testing studies, (a) for acute effects, (b) for subchronic/subacute effects, (c) for chronic effects?

33. Will it be required to duplicate the route of actual human exposure during future toxicity studies with animals?

34. Will it be possible to use non-inhalation toxicology data to predict human health hazards associated with inhalation exposures? Is this due primarily to economic constraints or is it likely to be a technically "acceptable" alternative?

35. Forecast potential advances in data evaluation and interpretation techniques that may permit improved communication of the results from toxicity testing to both decision makers and the general public (i.e. will the credibility of the scientific community to predict human health hazards improve, deteriorate or remain at its present level).

TR-477-14-14

REVIEW OF FORECASTS OF POTENTIAL TECHNOLOGY CHANGES THAT MAY IMPACT TOXICOLOGICAL TESTING REQUIREMENTS: GENERAL TOXICOLOGY

ICAIR Task Assignment No.: 107
Task Assignment Title: Impact of Changes

Task Assignment Report

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IMPACT OF CHANGES

Introduction

Having examined the supporting documents and the Candidate Questions/Issues For Forecasting Toxicological Testing Technology Advances (included as Appendix 1), the conclusion was reached that most of the authors did not respond to many of the questions. Thus, the decision was made to answer the candidate questions which dealt directly with general toxicology.

Answers to Candidate Questions/Issues for General Toxicology

5. Under the assumption that toxicological testing requirements will become more stringent (greater quality assurance), what will be the future value of toxicological data developed under present day standards?

   Most, if not all, scientific data has some value because it serves as a base for further development. Information developed 10, 15, or 20 years ago may not be statistically significant because insufficient numbers of animals were used in a particular test in comparison to today's standards, yet it still serves as a guide for today.

6. Will the centralized data base storage and retrieval mechanisms that are established or are being developed permit any significant reduction in toxicological testing requirements in the future?

   If one is interested in a substance that has been on the market or present in the environment for many years, then the system will be useful for basic information. On the other hand, if a substance is new then it is unlikely that the data retrieval system will reduce the need for toxicological tests to less than currently required for other substances. A reduction in the number of tests might be permitted for a substance if a structure-activity relationship could be established with a similar substance. It is doubtful, however, that the procedures would be reduced substantially.

7. What will be the future impact/role of epidemiology studies in human health assessments? (Will confirmatory human data always be required to supplement results from animal tests?)

   Epidemiology and human testing will always be involved if a chemical is distributed in the environment, but the manner of exposure will continue to be a key issue. Scientifically, one should collect a reasonable amount of data in laboratory animals with a particular chemical before considering human exposure. If the compound seems safe to use in animals and an appropriate safety factor is applied for human exposure, then some confirmatory human data could be collected. Perhaps the best way to collect such information would be to monitor subjects for many years after exposure. Aside from pharmaceutical compounds, most people are not exposed directly to chemicals, except in occupational exposure, but are exposed indirectly in some manner. Thus, there would not be any need to conduct direct tests on humans to simply confirm animal tests.

Epidemiological studies would be almost useless if studies were undertaken 10-15 years after introduction of the chemical into the environment. On the other hand, epidemiological studies could be developed early enough to be a major part of a well-planned monitoring program.

8. What will be the projected speed for acceptance of technology changes for toxicity testing in your specific discipline area?

   It takes many years to develop and test a new idea, especially in the complex field of toxicology. It also takes a long time for other scientists to test and verify your data. Thus, if a new idea were developed today and eventually proved to be an important tool, it probably would not be in major use for up to 8-10 years.

9. Forecast the level of support for toxicology research and development, especially in the area of basic research and its impact on technology.

   Considering the current political and economic conditions in the country, it seems likely that funds for toxicological research will either be reduced or remain about the same over the next several years. A general budgetary increase is not evident in the foreseeable future. Perhaps industry might increase its funds for basic research, if there happened to appear to be a marketing advantage. Clearly, the Federal Government dictates the direction of research through its funding programs and this is not likely to change in the near future.

10. What federal agency/organization programs are likely to be the pace setters for developing advances in toxicological testing technology?

    The National Toxicology Program and the National Institute of Health are currently the leaders in this field and will probably remain so in the future. The National Institute of Health is concerned primarily with purely basic research, whereas the National Toxicology Program focuses its attention on the more practical, technological matters. Unfortunately, neither organization is funded sufficiently to spawn major technological advances. The National Academy of Sciences does not conduct or sponsor research, so it could not be a pace setter. The Environmental Protection Agency is suffering from a lack of organization and is not considered a leader in the field of research.

11. Forecast the impact of mutagenic screening and other screening techniques on overall toxicology testing requirements.

    Certainly these techniques will have some impact on testing requirements, but it is unlikely that many new screening techniques will be developed in the near future. Screening procedures will be useful for some compounds where a definite positive or negative reaction is evident, but they will have only limited value when an intermediate response is obtained. Screening techniques may give guidance in some areas, but it is doubtful that they will change the way we now conduct toxicological tests.

12. What is the anticipated pace at which these screening techniques are likely to receive full acceptance as the basis for regulatory actions?

It seems unlikely that screening tests alone will ever be accepted as the entire basis for regulatory action, or at least they should not be. Acceptance by the scientific community may be slow as the techniques will have to be tested and retested to evaluate their validity.

13. Will concern for synergistic effects due to exposures to multiple chemicals significantly impact short- and/or long-term toxicological testing protocols?

It seems possible that if a person could be exposed to a variety of known chemicals on a regular basis, then similar exposure patterns should be developed and tested in laboratory animals. Sufficient information is now available on synergistic effects to suggest that enhancement of particular symptoms from exposure to multiple chemicals can occur. Thus, every precaution should be taken early in the preparation of testing protocols to take this into account.

14. Will there be a trend toward the consolidation of toxicology testing protocols?

No, in fact just the opposite may occur. For years, the trend has been to expand and add additional tests, and it seems likely that this trend will continue. In the early days of toxicology, tests with one species involving mostly acute exposures were routine. Today, usually two or more species are used and extensive acute, chronic and subchronic tests are employed. If the compound being examined is a suspected carcinogen, then more exhaustive procedures are used. The only reason that fewer tests would be conducted is that a highly reliable screening test could developed, and when it gave a positive response then there would not be any reason to proceed further.

15. What is/would be the impact of focusing on the toxicological properties of chemical groups as opposed to specific chemical compounds?

It is well-known that certain functional groups can have harmful toxicological properties and therefore should be viewed with caution. On the other hand, the mere presence of these functional groups does not necessarily guarantee a toxicological response. This suggests that knowledge of certain chemical groups is important, but that the development of toxicological protocols can not be focused entirely on such data. Perhaps with such knowledge, expansion of certain selected procedures may be called for, but employment of the routine toxicological tests should not be ignored.

17. Forecast the impact and pace of developments in improved risk assessment techniques.

All toxicological tests are designed and used to make risk assessments. Thus, the pace of development of new and expanded toxicological tests will govern the rate of improved risk assessment. When animal tests are extrapolated to man, the impact of risk assessment has considerable meaning. Frequently, individuals directly involved perceive risks and make risk assessments differently, depending upon the immediate circumstances. On the average, however, the general public is much more concerned about

the risk of getting cancer from a chemical, even though the chances are extremely low, than it is about the risk of being sterilized by a chemical when the chances are very high. Thus, it seems apparent that life threatening chemicals are perceived differently, even when the risk is low, than are chemicals that are not life threatening, but have a high incidence of physiological damage.

18. Forecast the role of structure activity relationships as they may replace certain toxicity testing requirements.

    If this were to occur, then the assumption must be made that all chemicals with similar structures are absorbed, metabolized, and excreted in the same manner. In some instances this is true, but in most it is not the case. It would be a convenient and easy way to classify chemicals and at the same time reduce the number of toxicity testing requirements. It is my opinion that we do not yet have a sufficient data base to make such judgements.

19. Forecast the role/impact of improved radiolabeling techniques and chemistry on toxicological testing requirements.

    The use of radiolabelled substances has had and will continue to have a profound impact on toxicological testing. The ability to detect picogram and nanogram quantities of toxic substances in body fluids, as well as environmental carriers, has advanced the field of toxicology immeasurably in the last decade and most certainly will be vital to the field in the next decade.

20. Evaluate the availability of laboratory animals on toxicology and/or requirements.

    A constant supply of all laboratory animals must be maintained regardless of any controversy or expense. If new chemicals are going to be synthesized, or various breakdown products and metabolites of old chemicals are to be studied, then testing must be done on animals.

22. Forecast the availability of scarce personnel on the ability to perform toxicity testing.

    Scarcity of personnel will continue to be a problem until more financial support by the government is put into the system. Universities are not expanding their departments that train toxicologists because of the lack of resources and in some instances because they do not have a good understanding of society's needs in this field. Another major problem is that universities have difficulty in maintaining qualified professors and support personnel because of the wide salary differential between universities and industry. It seems obvious and apparent that the personnel shortage will continue throughout the next decade unless a major national program is undertaken to lessen the problem. Likewise, the ability to perform appropriate toxicity testing will be greatly reduced.

23. Evaluate the role of scientific/professional societies in promoting technology changes that will impact testing requirements.

It seems apparent that professional societies will continue to play some part in testing requirements in the future. These organizations have made several excellent contributions in the past, but there seems to be considerable reservation on the part of government regulatory agencies to accept much help from "outsiders". The EPA has been especially slow to accept outside help. In this regard, there is a Congressional mandate that requires EPA to maintain a scientific advisory panel on pesticides, but it is quite apparent that the advice given by this panel is ignored on a regular basis. Of particular importance here is the fact that regulatory decisions are frequently made by government employees far less qualified than those appointed to the panel.

24. What will be the influence of current basic research investments on future testing technology?

Basic research always has some impact on applied research, such as testing technology, but it is difficult to assess this on a short-term basis.

26. Forecast the degree of concern for the safety and health of persons performing toxicity testing and what impact this will have on future testing resource requirements.

There is considerable concern now about the health of persons performing tests, and there are already strict government regulations now imposed for the Ames test and other toxicological facilities. This will tend to increase the cost of facilities, but it will also help to avoid any major health problems with employees. All facilities should be reviewed regularly and "up-dated" when it appears that a problem could exist.

30. What advances are likely in non-animal testing that will reduce the amount/extent of animal testing?

It is difficult to visualize any major changes in the way we conduct toxicological tests during the next 8-10 years. Many of the procedures we now use most likely will be refined and improved upon, but it seems doubtful that new "Ames tests" are forthcoming because of economic reasons, inadequately trained scientists, and lack of appropriate facilities. Aside from these reasons, it takes time to convince the scientific community of the usefulness of a particular procedure once it has been developed. For example, the "Ames test" for mutagenicity (and carcinogenicity) was developed several years ago, and was hailed at that time as a modern breakthrough for screening chemicals, but today many still question its reliability. Thus, it seems unlikely that toxicological tests currently in use today will differ substantially 10 years from now.

32. What technological changes are anticipated in the area of duration of toxicology testing studies, (a) for acute effects, (b) for subchronic/subacute effects, (c) for chronic effects?

It seems unlikely that any new developments will change the duration of the tests we now perform. These tests have been performed over and over again, and their reliability seems to be very good. I think that perhaps more emphasis will be on chronic tests as acute exposure is usually treatable, whereas chronic exposure can cause permanent and often lethal effects.

33. Will it be required to duplicate the route of actual human exposure during future toxicity studies with animals?

I believe that in most instances, this should be done. Even though I feel that the routine toxicological tests should be done, I also believe the "real" world should be approximated, by conducting similar animal exposure studies.

35. Forecast potential advances in data evaluation and interpretation techniques that may permit improved communication of the results from toxicity testing to both decision makers and the general public (i.e. will the credibility of the scientific community to predict human health hazards improve, deteriorate or remain at the present level).

I think communicating with the public about subjects such as this will always be a problem, especially if the information comes from the federal government. Even from my vantage point, I frequently have serious reservations about what the federal government says.

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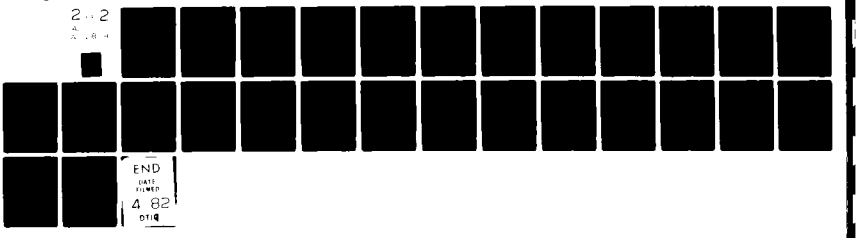

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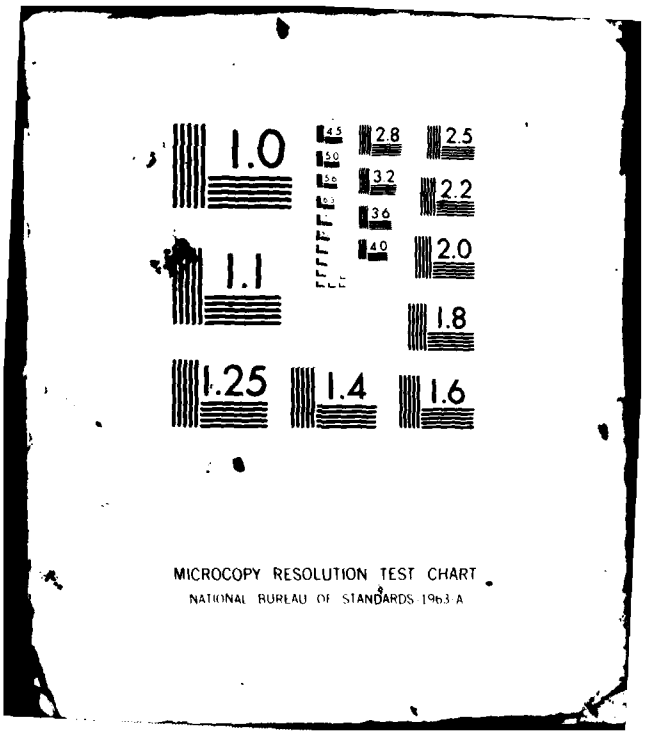
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NATIONAL BUREAU OF STANDARDS 1963-A

APPENDIX 1

CANDIDATE QUESTIONS/ISSUES FOR FORECASTING TOXICOLOGICAL TESTING TECHNOLOGY ADVANCES

1. Forecast equipment needs for laboratory toxicological testing.

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31. What advances are likely in non-animal testing that will reduce the amount/extent of animal testing?

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TR-477-14-15

REVIEW OF FORECASTS OF POTENTIAL TECHNOLOGY CHANGES THAT MAY IMPACT TOXICOLOGICAL TESTING REQUIREMENTS: MUTAGENICITY

ICAIR Task Assignment No.: 107
Task Assignment Title: Impact of Changes

Task Assignment Report

by

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March, 1981

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IMPACT OF CHANGES

Introduction

In my review of the consultants' Task Assignments Reports, I have concluded that their discussion has not focused on the mutagenicity tests. Therefore, a brief synopsis is given, emphasizing the impact of research advances on mutagenicity testing requirements, followed by the answers to candidate questions/issues that deal with mutagenicity tests.

The Predictive Value of Short Term Mutagenicity Tests

Most of the short-term mutagenicity tests widely used today, such as the Ames test, are designed to detect damage to DNA. DNA damage is now generally accepted as a major cause of cancer and genetic birth defects, and also may contribute to heart disease, aging, cataracts, and developmental birth defects. The correlation between the mutagenicity of chemicals and their carcinogenicity in animals has been verified by extensive testing of more than 300 chemicals. The Ames test alone gives 90 percent positive results for the tested chemical carcinogens and about 10 percent false positive results for the noncarcinogens. The few mutagenic chemicals that are known to be noncarcinogens may in fact be weak carcinogens that were not detected as such because of the statistical limitations of animal carcinogenicity tests. The positive results of a battery of mutagenicity tests are meaningful in predicting carcinogenicity and other animal toxicities of a chemical.

The significance of short-term mutagenicity tests in screening chemical carcinogens lies in their speed, low cost, and better precision and accuracy, and in the fact that epidemiological and animal cancer tests have their serious limitations. Limitations of epidemiology are related to the long latency period of carcinogenesis, multiple exposure to environmental factors, lack of controlled populations, incomplete data sources and the great expense. Limitations of animal cancer tests are related to the question of sensitivity due to statistical problems using small numbers or animals and the question as to how to extrapolate animal data to human responses. The cost of animal testing is also of concern ($250,000 per chemical for a test using two species of rodents in two sexes). These limitations make it impossible to test the 50,000 untested chemicals using epidemiological and/or animal cancer tests. The short-term mutagenicity tests therefore will play a vital role in the safety screening of these chemicals in the future.

Shortcomings of Mutagenicity Tests

Mutagenicity tests by design are not able to detect toxicants that do not act through a direct interaction with DNA; therefore, they are not capable of detecting epigenetic carcinogens such as asbestos and other promoters. A number of well established human carcinogens such as benzene, diethylstilbestrol, hydrazine, and some chlorinated hydrocarbons have not been detected as mutagens by the Ames test. These shortcomings of mutagenicity tests, however, are unlikely to discourage the increasing use of these tests, but rather, will be the stimuli of further research in the principles and practices of short-term genotoxicity tests. These shortcomings can also be corrected to some extent

by the use of a battery of tests to expand the testing scope, as is currently in practice.

Selection of Batteries of Tests

The use of a battery of short-term genotoxicity tests is largely determined by the spectrum of modes of actions of different chemicals which may be manifested in the component tests. The common tests currently in wide use are (in the order of increasing complexity): in vivo covalent binding to DNA, mutagenicity in bacteria, fungi, and insects, transformation of animal cells in culture followed by induction of tumors in animals by the transformed cells. This series of tests will likely be able to predict carcinogenicity of chemicals in animals. Tests designed to determine the effect of chemicals on germinal cells such as sperms are increasingly used to detect chemicals that cause genetic birth defects.

Within a single test, several operational parameters can be varied to alter the sensitivity of the test. In many cases a test needs to be performed for different classes of chemicals and under different sets of conditions to maximize sensitivity.

Laboratory Practices

There are about 2000 laboratories in the world which are currently performing some kind of mutagenicity test. Since chemicals being tested for mutagenicity or genotoxicity are potential carcinogens and teratogens, safety control to protect the laboratory personnel is of utmost importance. Recent advances in the technology of laboratory chemicals hoods have made this safety requirement easy to satisfy. The effectiveness in protecting laboratory personnel from occupational hazards in the short-term mutagenicity tests would be another factor contributing to the acceptance of these tests by regulatory agencies and industry.

In order to efficiently perform large number of tests involving different test systems and various sets of operational parameters, quality control and automation of facilities will inevitably become an important consideration. This means a requirement of larger capital investment in setting up the laboratory. It is anticipated that the best testing facilities will be associated with industrial operations and governmental testing centers rather than academic institutions. As safety and quality control regulations become more restrictive, there will be fewer laboratories that perform the testing routinely, but the scope of the tests will likely be greater than they are now.

The professionals in this field and laboratory personnel are relatively easy to train. Their training background may draw from a wide spectrum of disciplines. Unlike animal toxicity tests, there is little concern about not having enough pathologists to read the results of tests.

From the practical standpoint, short-term mutagenicity tests seem to be the most promising testing system that may be used to determine the toxicity and safety of the large number of chemicals that enter the human ecosystem.

Future Development

Because of the feasibility and the potential of short-term mutagenicity tests in detecting factors involved in the etiology of chronic disease, active research in this field is expected to continue and accelerate. Not only will the existing mutagenicity test systems be thoroughly characterized to maximize their sensitivity to different classes of chemicals, but also new test systems will be developed to expand the scope of detection. In addition, tests to measure other genetic end points such as sister chromatid exchanges, chromosomal aberrations, DNA repair, covalent binding to DNA, and cell transformation will also be used.

Active research is currently in progress in developing short-term tests for the detection of damage to target sites other than genetic components of cells. Effects of chemicals on the alveolar macrophages functions, leaking of marker enzymes from mammalian cells in culture, morphological changes in selected areas of the nervous system, and others have been developed into short-term tests of different degrees of sophistication. It is reasonable to predict that short-term tests will be developed for detection of harmful effects other than genetic effects of chemicals.

Answers to Candidate Questions/Issues

1. Forecast equipment needs for laboratory mutagenicity testing.

   With the higher demand in the safety and quality control of genetic toxicity tests, it is anticipated that a complete safety set-up to protect laboratory personnel will be required. For the handling of a large number of tests under proper quality control, some degree of automation in testing operation and certain electronic instruments to improve experimental measurements will likely be a feature of testing laboratories. The increased capital investment required will result in fewer laboratories being able to meet these standards, but the qualified laboratories will be able to do a better, more efficient job of testing chemicals.

2. Forecast changes in analytical chemistry required for support of mutagenicity test.

   One problem area in the mutagenicity testing is isolation and identification of mutagenic components in complex mixtures such as air, food, and smoke. Advances in separation techniques and identification methodology will greatly facilitate detection and measurement of environmental mutagens.

3. What is the impact of advances in statistical and mathematical approaches to mutagenicity testing requirements?

   Most short-term mutagenicity tests deal with a large population of cells and score rare events that take place in certain cells. The large numbers of "subjects" involved in the testing make statistical analysis of experimental results much easier.

4. What is the impact of computers and data acquisition equipment on toxicology testing?

Computer and data acquisition equipment is essential to the attempts to test the thousands of chemicals that still need to be tested. Considering the number of chemicals to be tested and the numerous variables to be optimized for each test, analysis of data by computers is the only means available to store all the data in a manageable manner so that duplications and errors may be minimized.

5. If toxicological testing requirements become more stringent in the future, what will be the future value of mutagenicity data developed today?

   The data developed under present day standards will still be valuable. They will provide the data base for comparison and for improvement in the right areas of toxicological testing.

6. Will the centralized data base storage and retrieval mechanisms that are established or being developed permit any significant reduction in mutagenicity testing requirements in the future?

   The centralized data base storage and retrieval mechanisms will not reduce mutagenicity testing requirements, but will prevent duplications and facilitate information retrieval such that any additional testing can be done in a more meaningful way and with improved experimental design.

7. What will be the future impact/role of epidemiology studies in human health hazard assessments? (Will confirmatory human data always be required to supplement results from animal tests?)

   Due to the increased acceptance of the short-term test results and the limitations of epidemiology studies, no significant increase in the frequency of epidemiological studies is anticipated. Studies will be done only on the few chemicals that have been shown to represent a tremendous health and economic impact. However, those studies chosen to be done have the benefit of better design and data sources available because of the ongoing advancements in toxicology and the continuous orderly accumulation of data.

8. What will be the projected speed for acceptance of technology changes for mutagenicity testing?

   The acceptance of technology changes for mutagenicity testing will be almost immediate because of the low cost and simplicity of the tests.

9. Forecast the level of support for toxicology research and development, especially in the areas of basic research and its impact on technology advancement.

   Increased support is anticipated, especially from industry, to push forward a good battery of short-term tests for the possible replacement of animal toxicity testing. The substantial savings in cost and time in developing new chemicals and the reduction of law suits related to their products are strong incentives for industry to support research in short-term tests.

10. What federal agency/organization programs are likely to be the pace setters for developing advances in mutagenicity testing technology, i.e. National Toxicology Program, EPA, National Academy of Sciences, etc.?

    Based on levels of support, the following agencies appear to have a great deal of interest in mutagenicity tests: NIOSH, NCI AND EPA.

11. Forecast the impact of mutagenic screening and other screening techniques on overall toxicology testing requirements.

    Mutagenic screening is already voluntarily accepted by industry as an essential preliminary toxicology test. I anticipate that short-term mutagenicity testing will become a major routine test to be performed on any new chemicals and any existing chemicals that have yet to be tested. Its impact on overall toxicology testing requirements is quite profound. It permits testing for a large number of chemicals; it reduces cost and time in the development of new and safer chemicals for consumer use; it helps prioritize animal and epidemiological studies of chemicals; it can be developed to possess definite values in predicting carcinogenicity, genetic birth defects and other health problems.

12. What is the anticipated pace at which these screening techniques are likely to receive full acceptance as the basis for regulatory actions?

    With continuous refinement and validation, it is anticipated that certain tests will receive full acceptance as the basis for regulatory actions in the near future. Positive results of these tests are toxicologically meaningful and will be taken very seriously by any regulatory agencies regardless of the legal status of the tests.

13. Will concern for synergistic effects due to exposures to multiple chemicals significantly impact short- and/or long-term toxicological testing requirements?

    Yes. Synergistic effects due to exposures to multiple chemicals is an area of challenge to genetic toxicologists and other toxicologists as well. Short-term mutagenicity testing is one of the few approaches that can be used to tackle this problem.

14. Will there be a trend toward the consolidation of toxicology testing protocols?

    Yes. The testing protocols have to be unified and standardized to minimize variations in the results of tests for the same types of toxicity.

15. What is/would be the impact of focusing on the toxicological properties of chemical groups as opposed to specific chemical compounds?

    Toxicological properties of chemicals are useful in determining effects or risk, whereas chemical properties are useful in determining environmental fate and exposure. Both are essential elements of toxicology testing. Neither should be neglected.

16. What progress related to cancer research would impact mutagenicity testing requirements?

Progress in the mode of action of genetic and epigenetic carcinogens will facilitate refinement or the development of short-term genotoxicity tests.

17. Forecast the impact and pace of developments in improved risk assessment techniques.

Awareness of the significance of environmental and occupational health problems will provide the incentive to keep a better record of the incidences and characteristics of diseases. Advances in pathological research will sharpen the diagnoses that shed light on the cause-effect relationship of diseases and exposure to chemicals. Better incidence data and diagnostic techniques will improve the accuracy of risk assessment.

18. Forecast the role of structure activity relationships as they may replace certain toxicity testing requirements.

Structure activity relationships determined by short-term tests are useful in estimating the relative potency of structurally related carcinogens without having to do animal tests for each chemical in question.

19. Forecast the role/impact of improved radiolabeling techniques and chemistry (analytical and clinical) on toxicological testing requirements.

Improved radiolabeling techniques will facilitate mutagenicity testing in the following ways:

a. Radio immuno assays to measure exposure to trace amounts of specific carcinogens.
b. Measurement of covalent binding of chemicals (radiolabeled) to DNA.
c. Toxicokinetic and metabolic studies to compare the fates of chemicals in different organisms.

20. Evaluate the availability of laboratory animals on mutagenicity testing technology and/or requirements.

Since the short-term mutagenicity tests do not require large numbers of laboratory animals, performance or development of these tests will not be affected by the availability of laboratory animals to any great extent.

22. Forecast the availability of scarce personnel on the ability to perform toxicity testing.

The availability of personnel capable of performing short-term tests should be a problem. The training background of the personnel can be drawn from a wide spectrum of scientific disciplines. There are no restrictions in enrolling in the training programs. The time required for training the mutagenicity testing technicians is also much shorter than the time required for training toxicologists or pathologists.

23. Evaluate the role of scientific/professional societies in promoting technology changes that will impact testing requirements.

Through their educational programs, regular meetings, and publications the scientific/professional societies in the area of toxicology will continue to facilitate identification of health problems caused by chemicals, stimulate communications among researchers to expedite technology transfer, and improve the understanding and application of mutagenicity tests. The activities of the following societies will likely be a driving force behind the rapid growth in the field of short-term mutagenicity testing: Society of Toxicology, Environmental Mutagenesis Society, American Cancer Society, and various task forces dealing with environmental carcinogens and mutagens.

24. What will be the influence of current basic research investments on future testing technology?

Current basic research investments will push forward our understanding of genetic toxicology and the designing of better genotoxicity tests. The money is well spent and the rewards will be rich.

26. Forecast the degree of concern for the safety and health of persons performing toxicity testing and what impact this will have on future testing resource requirements.

The safety and health of persons performing mutagenicity tests will continue to be of great concern because they are dealing with carcinogens or potential carcinogens. Hopefully a better understanding of toxicological principles and the availability of safety equipment will help eliminate some ungrounded fears over working with carcinogens. The substantial investments for safety equipment required for a mutagenicity testing laboratory will slow down the increase in the number of testing laboratories. See question #1 for further discussion.

31. What advances are likely in non-animal testing that will reduce the amount/extent of animal testing?

Advances in the following areas of non-animal testing will likely reduce the amount of animal testing:

a. Short-term tests using bacteria, fungi, and animal cells in culture as model biological systems.

b. Comparative pharmacokinetics and toxicokinetics of different classes of chemicals.

Combination of a and b may yield test results that are comparable or even better than those obtained by animal testing.

35. Forecast potential advances in data evaluation and interpretation techniques that may permit improved communication of the results from mutagenicity testing to both decision makers and the general public.

Advances in the understanding of the mechanisms of carcinogenesis will allow genetic toxicologists to evaluate and interpret the mutagenicity test results in terms of cancer and other more familiar effects. At the same time decision makers and the public will likely be better educated in toxicology through formal courses or news media. Increased use of the same terminology such as non-effect dose, threshold, dose-response relationship, etc. will serve to narrow the gap between the professionals and the laymen and improve their communication.

APPENDIX 1

CANDIDATE QUESTIONS/ISSUES FOR FORECASTING TOXICOLOGICAL TESTING TECHNOLOGY ADVANCES

1. Forecast equipment needs for laboratory toxicological testing.

2. Forecast changes in analytical chemistry required for support of toxicological testing.

3. What is the impact of advances in statistical and mathematical approaches to toxicological testing requirements (experimental design and data evaluation)?

4. What is the impact of computers and data acquisition equipment on toxicology testing?

5. Under the assumption that toxicological testing requirements will become more stringent (greater quality assurance), what will be the future value of toxicological data developed under present day standards?

6. Will the centralized data base storage and retrieval mechanisms that are established or being developed permit any significant reduction in toxicological testing requirements in the future?

7. What will be the future impact/role of epidemiology studies in human health hazard assessments? (Will confirmatory human data always be required to supplement results from animal tests?)

8. What will be the projected speed for acceptance of technology changes for toxicity testing in your specific discipline area?

9. Forecast the level of support for toxicology research and development, especially in the areas of basic research and its impact on technology advancement.

10. What federal agency/organization programs are likely to be the pace setters for developing advances in toxicological testing technology, i.e. National Toxicology Program, EPA, National Academy of Sciences, etc.?

11. Forecast the impact of mutagenic screening and other screening techniques on overall toxicology testing requirements.

12. What is the anticipated pace at which these screening techniques are likely to receive full acceptance as the basis for regulatory actions?

13. Will concern for synergistic effects due to exposures to multiple chemicals significantly impact short- and/or long-term toxicological testing requirements?

14. Will there be a trend toward the consolidation of toxicology testing protocols?

15. What is/would be the impact of focusing on the toxicological properties of chemical groups as opposed to specific chemical compounds?

16. What progress related to cancer research would impact toxicological testing requirements, i.e. in areas of defining specific causes for cancers, or in the treatment and "cure" of cancer?

17. Forecast the impact and pace of developments in improved risk assessment techniques.

18. Forecast the role of structure activity relationships as they may replace certain toxicity testing requirements.

19. Forecast the role/impact of improved radiolabeling techniques and chemistry (analytical and clinical) on toxicological testing requirements.

20. Evaluate the availability of laboratory animals on toxicology testing technology and/or requirements (controversies associated with use of dogs, scarcity and expense for use of primates, etc.).

21. Are advances/standardization of neurotoxicity and behavioral effects testing believed to take place in the relatively near future so that these types of effects will have greater acceptance as a basis for establishing rules and regulations?

22. Forecast the availability of scarce personnel (e.g. veterinary pathologists) on the ability to perform toxicity testing.

23. Evaluate the role of scientific/professional societies in promoting technology changes that will impact testing requirements.

24. What will be the influence of current basic research investments on future testing technology?

25. What are the best analogies to toxicity testing technology?

26. Forecast the degree of concern for the safety and health of persons performing toxicity testing and what impact this will have on future testing resource requirements.

27. What impact will medical treatment/advances have on reducing the concern with certain adverse toxic effects.

28. Will there be increased or decreased emphasis placed on toxicological effects which are reversible or irreversible?

29. What technology changes are anticipated in the areas of routes of exposure: (a) inhalation, (b) oral, (c) dermal, (d) ocular, (e) other?

30. What technology changes are anticipated in the area of animals used for toxicology testing, (a) rodents, (b) primates, (c) other animals?

31. What advances are likely in non-animal testing that will reduce the amount/extent of animal testing?

32. What technology changes are anticipated in the area of duration of toxicology testing studies, (a) for acute effects, (b) for subchronic/subacute effects, (c) for chronic effects?

33. Will it be required to duplicate the route of actual human exposure during future toxicity studies with animals?

34. Will it be possible to use non-inhalation toxicology data to predict human health hazards associated with inhalation exposures? Is this due to pimarily to economic constraints or is it likely to be a technically "acceptable" alternative?

35. Forecast potential advances in data evaluation and interpretation techniques that may permit improved communication of the results from toxicity testing to both decision makers and the general public (i.e. will the credibility of the scientific community to predict human health hazards improve, deteriorate or remain at its present level).

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REVIEW OF FORECASTS OF POTENTIAL TECHNOLOGY CHANGES THAT MAY IMPACT TOXICOLOGICAL TESTING REQUIREMENTS: INHALATION TOXICOLOGY

ICAIR Task Assignment No.: 107
Task Assignment Title: Impact of Changes

Task Assignment Report

by

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IMPACT OF CHANGES

Introduction

I will first attempt to answer some of the specific candidate questions/issues (listed in Appendix 1) as they impact on inhalation toxicology, then selectively critique some of the reports from the January 21st meeting convened by Life Systems, Inc. Finally, comments on aspects of the meeting summary that seem relevant to inhalation toxicology are included.

It is important to note that the assumption that state regulatory agencies will be dominated by federal laws retesting (and, by implication, setting of standards) is not true in California, where both OSHA and the State Air Resources Board set more stringent standards than the federal requirements and independently analyze the data base. This may have implications as to where toxicology research can be done (California's OSHA requires special facilities for work with asbestos at any level) and, perhaps, on where military manufacture can be done in the 1980's.

Answers to Candidate Questions/Issues for Inhalation Toxicology

Question No. 1

The most important technological change occurring from an analytical point of view is the tremendous increase in sensitivity and specificity of instrumental analysis over classical wet chemical techniques. Where previously mg levels of substances were required for detection and quantitation, ultraviolet and infrared spectroscopy have taken us to (10^{-6} g) levels; high-pressure liquid chromatography (HPLC), gas chromatography/mass spectroscopy, and atomic absorption spectroscopy to ng (10^{-9} g) levels; and flourescence detection coupled with HPLC to pg (10^{-12} g) levels of sensitivity for individual compounds. Future advances will feature increased use of microprocessors and mini-computers to automate such analytical instrumentation, and transfer of space-age devices for remote monitoring and telemetry of data for the analysis of inhaled substances. Inhalation toxicology can be automated at the current state of the art for any substance for which an assay exists that is adequately sensitive to monitor effluent streams from an exposure chamber. Future technology will focus on interfacing currently available detection instrumentation with generators via computers to allow such automation to be achieved at reasonable costs.

A further need is for components that will function reliably in such automated systems to allow sub chronic and chronic exposure regimens to be performed. For example, we currently perform exposures to ozone for up to 7 days using commercially available generators and chemiluminescent detectors interfaced by a medium-sized computer that require no human technicians other than to spot check equipment and to maintain animal hygiene. For inhalation toxicology testing of water-soluble substances, commercially available nebulizers can generate respirable aerosols (1 um particles), which can be monitored by optical particle counters (by light scattering) and by on-line chromatographic devices.

Improvements in devices and techniques for analysis of effluent streams and selectivity and reliability of such equipment is a necessary need for the next

decade to achieve the levels of automation currently practical with gaseous substances to be tested. At all levels (cost, protection of personnel, scientific of quality), such automation of exposure facilities is a prerequisite to embarking on an extensive program of testing substances via the inhalation route.

Question No. 5

If we ignore the political implications of GLP on university-generated research, I would agree with Wagner & Spencer that good data (and only good data from properly designed and executed experiments) will stand the test of time. I strongly disagree with Gittes that "all data have some value"; incorrect data may often be worse than no data at all. This problem is acute in inhalation toxicology, where the average quality of experimentation has been (and continues to be, in my opinion) shockingly poor. Unless quality and quantity of inhaled substances are rigorously controlled and monitored, unless health status of animals (especially with regard to bacterial and/or mycoplasmal pneumonias) is rigorously monitored, unless experimental design is rational (multiple dose regimens, some of which are sublethal), and unless appropriate end points for assay of effects are chosen, such experimental data may be of no value. Perhaps due to the cost of such testing, many of the published data are from experiments that have not adhered to such minimal standards of quality assurance.

Question Nos. 7, 11, 31 and 34

The most important technological change currently occurring from a biological point of view is the extensive evaluation of in vitro screening techniques as potential supplementary or replacement tests for in vivo animal (or human) exposures. One important goal of any future prediction exercise ought to be an estimation of the extent that standard animal toxicology testing can be replaced by such in vitro tests from both a technological and a political point of view. If we focus on inhalation toxicology, we can break this question into two parts: carcinogenicity (cancer causing potential) and other effects (upon the lung or systemic). My own prejudice is that we will come scientifically to accept the Ames assay (and any future refinements) as a red flag, such that positive mutagenic compounds will be dropped from use or consideration for further development if non-mutagenic alternatives are available. I do not think society (industry, politicians, special interest groups, public) as a whole will ever be able to understand risk in quantitative terms, so there will be strong political pressure in this direction. Negative results in Ames tests or tissue culture experiments will not convince scientists (or the public) to bypass animal testing for carcinogenicity unless we know a lot more about mechanisms and empirical relations of cancer causation then we presently do. That leaves the problem of what we will do with mutagenic compounds that do not offer suitable alternatives for their use and are deemed necessary (e.g. diesel fuel for smoke screens in the battlefield). My guess is we will continue standard animal testing, continue to have bitter controversies over weak carcinogens such as cyclamate and saccharin, and not advance in this area as the problems tend to be politically dominated rather than "needing more data to get a scientific answer". Simplistic statements such as that of Gittes (p. 1) that "a change in the absolute nature of the Delaney Clause would not affect the toxicological requirements for safety testing" are total

non-sequitors in a real-life scenario where both Congress and the FDA violate or enforce the law at their discretion (e.g. nitrites, cyclamates) based on perceived public response rather than the law as written.

When we deal with in vitro screening tests to replace or supplement inhalation toxicology with respect to areas other than carcinogenesis, we have a field in its infancy that should be actively supported in the next decade. Spencer discusses one system in his report. More importantly, basic research presently underway on ion transport by epithelial cells and the roles of calcium ion flux and calmodulin in such cells promises to ultimately explain epithelial cell irritation in molecular terms. Such insights should allow rational development of simple in vitro systems to screen for acute toxicity of substances that affect airway epithelial cells, and provoke asthmatic attacks, coughing and a variety of other undesirable responses (and possible long-term damage) in exposed individuals. Since the vast majority of physiological pulmonary function testing sees only these same types of effects in animals, I foresee replacement of animal toxicity testing with physiological end points by such in vitro testing beginning to occur in the 1980's. I agree with Spencer that we will not be willing to replace histopathology with in vitro testing in the foreseeable future for routine animal toxicological experimentation.

Human epidemiology is another field (especially from the inhalation toxicology - air pollution point of view) that has suffered in the past from a lot of bad science done by less than optimally competent investigators. In this era of easy access to computers, there is no excuse for doing bad (or even mediocre) epidemiology. Yet we lag behind Great Britain in this area because of our lack of centralized medical records (and data) for following large cohorts of exposed individuals over time. In no area is the United States Army better able to use its existing resources to improve its toxicological surveillance than this, in my opinion. I would suggest that computerized data be kept (classified if desirable to do so for privacy or security reasons) on all known or suspected exposures of all soldiers to toxic, suspected toxic, or esoteric (as compared to the civilian environment) compounds during their military service. Such data will allow ready retrospective surveillance for excess morbidity and/or mortality association with specific compounds via the National Death Index, V.A. hospital records, and the Army's own system of medical facilities. Most of the scientific problems with retrospective epidemiological studies can be avoided by careful prospective data acquisition. I think Rothman is unduly pessimistic as to what role epidemiology ought to play in the toxicology of the 80's and 90's.

In general, I see inhalation toxicology as being essential to generate data to predict human health hazards associated with inhalation exposures for some time to come. The lung is an extremely active organ metabolically and can not be ignored as a source/cause of systemic toxicity beyond the lung due to inhalation exposures. For example, excess bladder cancer in cigarette smokers (Rothman, Figure 1) is thought to be due to enzymes in the lung epithelial cells that metabolize compounds in cigarette smoke to potent water soluble carcinogens that reach the bladder via the bloodstream, whereby they exert their cancer producing effect. Thus, the lung is not merely a portal of entry, but is also, like the liver, a metabolic factory whose contribution to xenobiotic metabolism can not be ignored. The role(s) of the various highly

sensitive instrumental assays discussed above in stimulating research in metabolism by the lung of trace amounts of inhaled xenobiotics ought not be ignored in the 1980's; availability of pure compounds and widespread familiarity with radioimmunoassay techniques may extend the detection limits for specific compounds to the fg (10^{-15} g) level. As a result, trace contamination of substances with potentially toxic impurities will become a major concern (e.g. dioxin in "Agent Orange") for toxicology. As analytical techniques become more sensitive, purity becomes a relative, rather than absolute, concept. This will open a vast new Pandora's box of compounds that will be perceived as requiring toxicological evaluation.

Critique of Reports

I agree with Spencer's statement that behavioral toxicology is being (prematurely) strongly encouraged by EPA. My perception of the field is that it is experimental pyschology under a new title and has not been documented as yet to assay appropriate end points to serve as toxicological indexes. These are attractive experiments to perform because they are cheap, easy, and can be done by semi-skilled technicians. I would not heed these arguments by proponents until enough basic research has been done to document their validity as predictors of such assays, and I would not allow myself to be seduced by an attractive jargon that substitutes words and phrases for complex and poorly understood behaviors. Spencer and I obviously agree on the importance of publication in peer-reviewed journals. We also agree on the fact that all investigators do not perform good experiments, and proper experimental design and evaluation techniques are a prerequisite to achieving credible data.

The role of synergism and potentiation due to exposure to multiple chemicals is an especially relevant concern to inhalation toxicology, both from an occupational exposure perspective and from the point of view of further contamination of the polluted ambient air we breath as civilians. Examples of synergistic effects in inhalation toxicology are well documented: SO_2, which is very soluble in water, is efficiently removed by the nose during normal breathing. SO_2 is much more toxic in the presence of respirable aerosols (less than 5 um diameter particles) or particles, as it can "piggyback" past the nasal defenses to penetrate into the deep lung. SO_2 is also apparently more dangerous in the presence of strong oxidants (ozone, NO_2), due to chemical reactions occurring that form more toxic aerosols of sulfuric acid or related compounds.

The current ambient air quality standards are based on a hodgepodge of epidemiological data, animal experiments, controlled (acute) human exposures, and questionable statistical inferences as to thresholds (usually) based on pulmonary function (physiological) tests. Regulations are all based on single substance experiments and criteria. Attention is currently being focused upon health effects of "mixed pollutants", a term appropriated from epidemiology suggesting that the whole urban atmosphere is more toxic than the sum of its parts. Funding agencies are urging the study of, and scientists at present are studying, mixtures. Regulatory agencies in the 80's should respond to these pressures and are likely to attemp to coordinately regulate classes of substances based on real or postulated synergistic effects. Spencer (p. 6) singles out the petroleum industry as especially concerned via petrochemicals; the synthetic fuels

industry is also heavily committed to this area of concern. Identification of promoters and/or co-carcinogens is a difficult task of obvious relevance to these concerns.

Meeting Summary Aspects

1b-"The baseline animal for mammalian tests is the mouse."

In inhalation toxicology (except perhaps for LD_{50} determinations), the baseline animal is the rat, for several reasons above and beyond tradition. Rats are commercially available in large quantities, bred and raised in barrier facilities that allow them to be free (ideally) of bacterial or mycoplasmal lung infections, a necessity for toxicological evaluation by the inhalation route. Viruses in the lungs are endemic and an unsolved (usually ignored) problem. At present, no other animal of comparable size with clean lungs is commercially available in quantity. The size of an animal and its lung are important if one wishes to do pulmonary physiology or biochemical evaluations on single animals with current techniques. Genetic homogeneity is another important consideration; inbred strains allow for replication of experiments in different laboratories. I foresee some replacement of rats with mutant mice to model specific at-risk human groups for selected inhalation exposures (genetically obese, hypertensive, and/or immunosuppressed mice, for example) in the future, but any workup beyond body count and routine histopathology will require use of rats or larger animals until new techniques are validated. As sub-chronic and chronic exposures are performed, the value of each animal will become high enough to demand increased sophistication of workup. Acute tests such as LD_{50} measurements or perturbations of respiratory rate may continue to be performed in mice, but correlation with chronic rat experiments will present problems in this scenario.

2d-"Baseline number of doses in dose level response tests is 2 plus control."

Two is not enough. Since two points make a straight line and the concept of linear extrapolation to the origin is controversial, to say the least, the baseline number of doses ought to be 3 (or more), with minimal or no lethality at the highest dose to prevent inadvertant selection of a non-random subpopulation (the survivors).

3-"Inhalation chamber design . . ."

Room-like chambers are expensive and probably unnecessary for any but the most risky of test compounds. Use of negative pressure in the chambers to prevent leakage to the exterior during sampling and careful scrubbing of effluent streams with baghouse activated charcoal, and/or HEPA filters prior to release to the atmosphere should be adequate for most gases and particulates.

Due to the costs of developing and equipping such facilities, large centralized facilities with high technology are probably much more versatile and cost effective than small, decentralized facilities. Consideration should be given to developing such a facility with either extramural access to bring in out-of-house expertise, or with civilian oversight using the universities as a potential resource. A prototype for this concept is the Administration of the Air

Force's Toxic Hazards Research Laboratory at Wright Patterson Air Force Base in Dayton, Ohio, by the University of California, Irvine. Since the appropriate role of the universities is the generation of new knowledge rather than technical data per se, such arrangements allow facilities to acquire and maintain state-of-the-art expertise on a contractual basis, while retaining in-house control of program, quality assurance and personnel.

4b-"Use of core analytical facilities . . ."

See comments directly above (3). Again, centralized facilities for high technology instrumentation make sense and again, extramural management by a university to attempt to prevent instant obsolescence is highly recommended.

5-"Solid waste disposal techniques (incineration) . . ."

Can this function be linked to electric power production (cogeneration) to make it more cost effective and more tightly controlled?

2b-"Epidemiology: Decreasing availability of other data. . ."

Could service experience be linked to VA records more effectively than at present to create a resource rather than a problem (vis-a-vis privacy)?

2-"Economics: Cost of toxicology testing . . ."

The cost of $500,000 - $750,000 for two species for long-term testing of a compound seems high to me, even for inhalation toxicology, which is the most expensive modality. It need not cost this much. What assumptions were made about indirect costs and test volume to arrive at these numbers? Were industrial testing costs used (overhead, higher salaries than military, amortization of facility over a single or a few compounds, profit) or were true costs evaluated? Certainly such testing could be done at a university, via a contract, for a fraction of that cost were the facility available. Careful use of consultants, at all stages of contract formulation, is urged if this approach is adopted; inhalation toxicology can probably be performed properly, rigorously, and efficiently at a reasonable cost in properly designed, built and administered facilities.

APPENDIX 1

CANDIDATE QUESTIONS/ISSUES FOR FORECASTING TOXICOLOGICAL TESTING TECHNOLOGY ADVANCES

1. Forecast equipment needs for laboratory toxicological testing.

2. Forecast changes in analytical chemistry required for support of toxicological testing.

3. What is the impact of advances in statistical and mathematical approaches to toxicological testing requirements (experimental design and data evaluation)?

4. What is the impact of computers and data acquisition equipment on toxicology testing?

5. Under the assumption that toxicological testing requirements will become more stringent (greater quality assurance), what will be the future value of toxicological data developed under present day standards?

6. Will the centralized data base storage and retrieval mechanisms that are established or being developed permit any significant reduction in toxicological testing requirements in the future?

7. What will be the future impact/role of epidemiology studies in human health hazard assessments? (Will confirmatory human data always be required to supplement results from animal tests?)

8. What will be the projected speed for acceptance of technology changes for toxicity testing in your specific discipline area?

9. Forecast the level of support for toxicology research and development, especially in the areas of basic research and its impact on technology advancement.

10. What federal agency/organization programs are likely to be the pace setters for developing advances in toxicological testing technology, i.e. National Toxicology Program, EPA, National Academy of Sciences, etc.?

11. Forecast the impact of mutagenic screening and other screening techniques on overall toxicology testing requirements.

12. What is the anticipated pace at which these screening techniques are likely to receive full acceptance as the basis for regulatory actions?

13. Will concern for synergistic effects due to exposures to multiple chemicals significantly impact short- and/or long-term toxicological testing requirements?

14. Will there be a trend toward the consolidation of toxicology testing protocols?

15. What is/would be the impact of focusing on the toxicological properties of chemical groups as opposed to specific chemical compounds?

16. What progress related to cancer research would impact toxicological testing requirements, i.e. in areas of defining specific causes for cancers, or in the treatment and "cure" of cancer?

17. Forecast the impact and pace of developments in improved risk assessment techniques.

18. Forecast the role of structure activity relationships as they may replace certain toxicity testing requirements.

19. Forecast the role/impact of improved radiolabeling techniques and chemistry (analytical and clinical) on toxicological testing requirements.

20. Evaluate the availability of laboratory animals on toxicology testing technology and/or requirements (controversies associated with use of dogs, scarcity and expense for use of primates, etc.).

21. Are advances/standardization of neurotoxicity and behavioral effects testing believed to take place in the relatively near future so that these types of effects will have greater acceptance as a basis for establishing rules and regulations?

22. Forecast the availability of scarce personnel (e.g. veterinary pathologists) on the ability to perform toxicity testing.

23. Evaluate the role of scientific/professional societies in promoting technology changes that will impact testing requirements.

24. What will be the influence of current basic research investments on future testing technology?

25. What are the best analogies to toxicity testing technology?

26. Forecast the degree of concern for the safety and health of persons performing toxicity testing and what impact this will have on future testing resource requirements.

27. What impact will medical treatment/advances have on reducing the concern with certain adverse toxic effects?

28. Will there be increased or decreased emphasis placed on toxicological effects which are reversible or irreversible?

29. What technology changes are anticipated in the areas of routes of exposure: (a) inhalation, (b) oral, (c) dermal, (d) ocular, (e) other?

30. What technology changes are anticipated in the area of animals used for toxicology testing, (a) rodents, (b) primates, (c) other animals?

31. What advances are likely in non-animal testing that will reduce the amount/ extent of animal testing?

32. What technology changes are anticipated in the area of duration of toxicology testing studies, (a) for acute effects, (b) for subchronic/subacute effects, (c) for chronic effects?

33. Will it be required to duplicate the route of actual human exposure during future toxicity studies with animals?

34. Will it be possible to use non-inhalation toxicology data to predict human health hazards associated with inhalation exposures? Is this due primarily to economic constraints or is it likely to be a technically "acceptable" alternative?

35. Forecast potential advances in data evaluation and interpretation techniques that may permit improved communication of the results from toxicity testing to both decision makers and the general public (i.e. will the credibility of the scientific community to predict human health hazards improve, deteriorate or remain at its present level).

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